ISSN 2220-315x (online)

# World Journal of *Experimental Medicine*

Quarterly Volume 14 Number 3 September 20, 2024

Published by Baishideng Publishing Group Inc

World Journal of Experimental Medicine

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## ABOUT COVER

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*WJEM* mainly publishes articles reporting research results and findings obtained in the field of experimental medicine and covering a wide range of topics including clinical laboratory medicine (applied and basic research in hematology, body fluid examination, cytomorphology, genetic diagnosis of hematological disorders, thrombosis and hemostasis, and blood typing and transfusion), biochemical examination (applied and basic research in laboratory automation and information system, biochemical methodology, and biochemical diagnostics), *etc*.

## INDEXING/ABSTRACTING

The *WJEM* is now abstracted and indexed in PubMed, PubMed Central, Scopus, Reference Citation Analysis, China Science and Technology Journal Database, and Superstar Journals Database. The *WJEM*'s CiteScore for 2023 is 1.7 and Scopus CiteScore rank 2023: Internal medicine is 109/167.

## RESPONSIBLE EDITORS FOR THIS ISSUE



**NAME OF JOURNAL**
*World Journal of Experimental Medicine*

**ISSN**
ISSN 2220-315x (online)

**LAUNCH DATE**
December 20, 2011

**FREQUENCY**
Quarterly

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**EXECUTIVE ASSOCIATE EDITORS-IN-CHIEF**
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**EDITORIAL BOARD MEMBERS**
https://www.wjgnet.com/2220-315x/editorialboard.htm

**PUBLICATION DATE**
September 20, 2024



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Department of Clinical Laboratory, The Affiliated Suzhou Hospital of Nanjing Medical University, Suzhou Municipal Hospital

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*World J Exp Med* 2024 September 20; 14(3): 91739

DOI: 10.5493/wjem.v14.i3.91739

ISSN 2220-315x (online)

EDITORIAL

# Malignant pleural mesothelioma: The disdained member of thoracic oncology!

Divya Khosla, Pawan Kumar Singh, Bharath A Chhabria, Vaishali Kataria, Navneet Singh, Rakesh Kapoor

**Specialty type:** Oncology

**Provenance and peer review:** Invited article; Externally peer reviewed.

**Peer-review model:** Single blind

**Peer-review report's classification**

**Scientific Quality:** Grade B
**Novelty:** Grade B
**Creativity or Innovation:** Grade B
**Scientific Significance:** Grade B

**P-Reviewer:** Sun GH

**Received:** January 3, 2024
**Revised:** July 17, 2024
**Accepted:** July 30, 2024
**Published online:** September 20, 2024
**Processing time:** 238 Days and 11.9 Hours

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## Abstract

Pleural mesothelioma is a very aggressive malignancy that arises from the pleural mesothelial cell lining and is linked strongly to prior asbestos exposure. The ban on asbestos has helped to lower the incidence, but in developing countries like India, it is expected to rise. It has an extended latency period usually progressing over decades and presents with nonspecific symptoms. It has a median survival ranging between 10-22 months. The diagnosis of malignant pleural mesothelioma is challenging and is done using computed tomography (CT), magnetic resonance imaging, or positron emission tomography-CT, with the last two predicting the resectability of the tumor better than CT alone. A pleural biopsy along with an array of immunohistochemical markers, such as p16, BRCA1 associated protein 1, and claudin-4, are required for a definitive diagnosis. Several genetic alterations have prognostic significance as well. The current histological subtype identification is indispensable for decision making because of the new therapeutic avenues being explored. The combination of nivolumab and ipilimumab-based immunotherapy outperformed platinum and pemetrexed-based chemotherapy in terms of survival benefit and improved quality of life especially for non-epithelioid subtypes. However, the latter continues to be a robust treatment option for patients with the epithelioid subtype. Surgery is recommended for resectable cases with radiotherapy being indicated in neoadjuvant, adjuvant, and palliative

settings along with systemic treatment. This review article provides an overview of epidemiology, etiology, clinical manifestations, diagnostic approaches (including immunohistochemical and genetic markers), staging, and multidisciplinary approaches to current treatment for malignant pleural mesothelioma using surgery, chemotherapy, immunotherapy, and radiotherapy. It also sheds light on some recent studies (EMPHACIS, CALGB30901, Checkmate-743, *etc.*) that have led to significant developments in recent years with clinically meaningful results.

**Key Words:** Chemotherapy; Diagnosis; Immunotherapy; Malignant pleural mesothelioma; Radiotherapy



**Core Tip:** This review article highlights the importance of preventive measures, early and accurate detection using imaging, histopathological markers, and multidisciplinary approaches for the treatment of malignant pleural mesothelioma using surgery, systemic chemotherapy, immunotherapy, and radiotherapy. Recent novel developments in systemic treatment have expanded the therapy armamentarium, and more work needs to be done in order to frame tailored therapies for this aggressive tumor presenting in late stages and associated with a poor prognosis due to limited treatment options. It is also essential to translate these evolving treatment options into increased overall survival along with improved quality of life.

**Citation:** Khosla D, Singh PK, Chhabria BA, Kataria V, Singh N, Kapoor R. Malignant pleural mesothelioma: The disdained member of thoracic oncology! *World J Exp Med* 2024; 14(3): 91739
**URL:** https://www.wjgnet.com/2220-315x/full/v14/i3/91739.htm
**DOI:** https://dx.doi.org/10.5493/wjem.v14.i3.91739

## INTRODUCTION

Mesotheliomas are aggressive neoplasms arising from the serosal surface of the pleural cavity, peritoneal cavity, pericardium, or the tunica vaginalis of the testis. Malignant pleural mesothelioma (MPM) is the most common, contributing to 80% of all mesotheliomas. Occupational asbestos exposure is regarded as the single most important etiological factor in the development of mesothelioma. However, environmental exposure to asbestos, radiation, and other mineral exposures are beginning to emerge as important risks. In addition, MPM poses a significant treatment challenge with 5-year survival being as low as 5%-10%[1]. With an increase in awareness regarding the ill effects of asbestos and stringent laws banning the use of asbestos, there has been a decline in the incidence of MPM in the West. However, continued and unregulated use of asbestos in low-income and middle-income countries is likely to significantly increase the mesothelioma burden in the coming years.

## EPIDEMIOLOGY

The incidence of MPM varies and is difficult to document in countries (like India) without registries. The age adjusted annual incidence of MPM in the United States was about 9.8 cases per million[2]. In the United States, the incidence peaked around the late 1990s to early 2000s and declined thereafter owing to the control of asbestos mining and use[2,3]. The trend is similar in other developed nations like the United Kingdom and Australia. However, due to the rampant use of asbestos in developing nations and continued mining in a few countries, the incidence of mesothelioma is predicted to increase several fold in the coming years. Although India has taken measures to curb the mining of asbestos, use of asbestos imported from Russia, Kazakhstan, China, and Canada continue[4]. Asbestos is utilized in cement pipes, plumbing, roofing of homes, insulating heavy machinery, shipbuilding and breaking, and brake lining[4]. India tops the list of countries with maximum asbestos usage of 318000 metric tons[5]. Asbestos exposure resulting in MPM has also been reported in household contacts of asbestos miners. In addition, environmental exposure also increases the risk of MPM[6]. Given the sex differences due to the occupational exposure, MPM remains more common in males when compared to females[2].

## RISK FACTORS

Inhaled asbestos exposure (either occupational, para-occupational, or environmental) is the foremost cause of MPM. Asbestos is a fibrous silicate of various chemical types. It exists in two forms, amphibole and chrysotile with the latter having a lower carcinogenic potential. MPM has a long latency period of approximately 20 years to 50 years[7]. The incidence following asbestos exposures increases linearly with the intensity of the exposure and exponentially with the duration of exposure[5].

Radiation exposure in the treatment of other malignancies accounts for subsequent MPM development, again with a long latency period[8]. Exposure of nuclear radiation has also been implicated to increase MPM risk. Patients developing MPM after radiation exposure are different from those with MPM due to asbestos exposure. They are generally younger and have a significantly longer overall survival[9]. Certain germline variants have been observed in MPM. Mutations in the gene encoding BRCA1 associated protein 1 (*BAP1*) has been shown to accelerate development of MPM in those exposed to asbestos[5]. Variants in DNA repair genes like *PALB2*, *BRCA1/2*, and cyclin-dependent kinase inhibitor 2A ( *CDKN2A)* also accelerate the development of MPM[5].

## CLINICAL PRESENTATION

The majority of patients present with nonspecific symptoms when they are in the sixth or seventh decade, several years after the exposure to asbestos has ceased. Symptoms include progressive dyspnea, dry cough, and chest pain. The symptoms may be attributable to the pleural effusion or volume contraction associated with the disease. Chest pain may or may not be pleuritic in nature and is usually due to the local invasive nature of the tumor leading to bone erosion or nerve impingement. Fatigue, anorexia, weight loss, sweats, and malaise are noted as the disease progresses. Distant metastasis is rare, while local invasion is known. Physical findings correspond to pleural effusion early in the disease, but ipsilateral volume contraction with rib crowding and scoliosis become apparent later in the disease course. Findings such as palpable nodules (at previous thoracocentesis puncture sites) may be seen.

Radiological imaging may include a frontal chest X-ray that is done as a part of the initial evaluation of symptoms. However, contrast-enhanced computed tomography (CT) of the chest and upper abdomen is recommended as the initial method of investigation[5]. Common chest X-ray findings include varying volumes of pleural effusion with or without pleural thickening, pleural mass, plaques, and calcification. Volume loss with ipsilateral mediastinal shift may also be seen. Advanced disease may demonstrate mediastinal extension with widening. Radiological features of asbestosis (bilateral basal fibrosis) are seen in only 20% of cases of MPM.

CT findings (in addition to pleural effusion) include pleural thickening, which may be seen in 90%-92% of the cases in some form. The pleural thickening may be circumferential, but focal thickening with nodules, loculated effusion, and mediastinal pleural thickening are more suggestive of mesothelioma[10].

Magnetic resonance imaging (MRI) is another modality that may be used in assessment. MRI has exhibited marginally higher sensitivity than CT for predicting resectability at the diaphragm and chest wall (100% *vs* 93%-94%, respectively) and has shown better accuracy in detecting solitary foci of chest wall invasion, endothoracic fascia involvement and brachiocephalic vessels involvement[11]. However, with the widespread use of positron emission tomography with computed tomography (PET-CT), which provides information on the metabolic activity along with anatomical extent, the use of MRI is now restricted to those cases who require additional soft tissue evaluation prior to surgery and in whom the use of iodinated contrast agents is contraindicated. Several series have suggested that PET-CT imaging is the most reliable imaging modality for initial assessment, particularly in determining whether a tumor is resectable[12].

## DIAGNOSIS

The clinico-radiological presentation guides histopathological or cytological confirmation of the diagnosis. Pleural fluid cytology and immunocytochemistry is an accepted modality for diagnosing if cytopathology expertise is available. The sensitivity ranges between 30%-75% and specificity between 80%-99%[13], while an Indian study reported a sensitivity and specificity of 75% and 96%, respectively[14].

Biopsies are obtained percutaneously under image guidance during medical thoracoscopy or during video-assisted thoracoscopic surgery. Thoracoscopy is the preferred technique and allows extensive inspection of the pleura. Multiple, large biopsies that include subpleural tissue for the histological assessment of invasion can be obtained. Medical thoracoscopy can diagnose MPM with a sensitivity of 88%-100% and specificity of 100%[15]. The presence of local invasion, pleural thickening, absence of pleural effusion, and volume loss often make thoracoscopic biopsy challenging. In patients with advanced disease, bronchoscopic modalities including endobronchial ultrasound maybe used to obtain tissue[16].

## SUBTYPES AND STAGING IN PLEURAL MESOTHELIOMA

### *Histological subtypes*

Genomic analysis and prospective data from the past decade have significantly improved the understanding of pleural mesothelioma. Following these advances, the World Health Organization has updated their original classification system in 2021[17]. Overall, pleural mesothelioma has been classified based on the extent of involvement. Localized mesothelioma requires diagnosis based on imaging, surgical resection, and histology. Pathological evaluation must rule out any evidence of invasion along with confirmation of mesothelial origin using immunohistochemical (IHC) markers[18]. The previously described "well differentiated papillary mesothelial tumor" type has been renamed to well differentiated papillary mesothelioma and removed from the malignant group due to its benign course and better prognosis.

In the latest update, mesothelioma in situ has also been described as a distinct entity. It is identified based on their molecular features: Loss of BAP1 or MTAP on IHC or CDKN2A deletion on fluorescence in situ hybridization[19]. In addition to these features, invasion must be ruled out to call it mesothelioma in situ. Mesothelioma can be either localized or diffuse. The diagnosis of localized mesothelioma is often reached upon multidisciplinary discussion. Diffuse pleural mesothelioma is further classified into epithelioid, sarcomatoid, or biphasic. The World Health Organization classification stresses upon the need of good tissue for the diagnosis, achieved using medical thoracoscopy, video-assisted thoracoscopic surgery, or image-guided biopsies. Diagnosis revisions are also advised based upon resection specimens.

The epithelioid subtype is the most common subtype, representing nearly 80% of all cases. The epithelioid variant is associated with better prognosis compared to the other subtypes. Within the epithelioid morphology, it is recommended to describe the architectural patterns that might have prognostic significance (solid and micropapillary features have poorer prognosis). The report must also include the grade of differentiation (high or low) based on nuclear atypia, mitoses, and necrosis.

The sarcomatoid subtype is characterized by the presence of desmoplastic reaction with or without spindle cells arranged in sheets. Sarcomatoid tumors are associated with a distinctly poor prognosis. When compared with the epithelioid variant, sarcomatoid tumors are not commonly associated with pleural effusion.

The biphasic subtype is characterized by the presence of both types of tumor cells. In resected specimens, it is recommended to report the individual component of cells with epithelioid and sarcomatoid characteristics. Biphasic tumors must have greater than 10% of either feature. However, in smaller biopsies such qualification has been found to be difficult and is not considered mandatory. However, the prognosis is driven mainly by the proportions of the sarcomatoid component in such cases[20].

### *IHC features*

Reporting of mesothelial tumors must include IHC workup to confirm the mesothelial origin of such tumors. IHC markers of common cancers with frequent pleural metastasis must be done based on suspicion, including lung (TTF1 and napsin for adenocarcinoma, p63 for squamous cell carcinoma), breast, ovarian, and colorectal cancers. In addition to the diagnosis and differentiation of such (especially epithelioid) tumors from other metastatic cancers, these markers are also useful in predicting prognosis[21]. However, none of the makers have absolute sensitivity or specificity, which mandates the use of an array of such markers. In a 2021 update, claudin 4 has been highlighted as a negative IHC marker with high diagnostic accuracy to differentiate mesothelioma from other metastatic carcinomas. Additionally, the European Respiratory Society/European Society of Thoracic Surgeons/European Association for Cardio-Thoracic Surgery/European Society for Radiotherapy and Oncology guidelines have recommended the use of CDKN2A (p16) deletion (using fluorescence in situ hybridization, commonly found in sarcomatoid variants) and BAP1 (using IHC, commonly demonstrated in epithelioid variants) for further characterization of the tumors[22]. In a 2021 update, loss of MTAP expression using IHC was found to correlate well with CDKN2A deletion and can be used as a surrogate. These markers have been demonstrated to have acceptable accuracy in cytological specimens as well.

Evaluation of tumor immune microenvironment has also been analyzed and shown to have a linear relationship between PDL1 expression and sarcomatoid differentiation leading to an overall poor prognosis in tumors with increased PDL1 expression. Non-epithelial tumors are also characterized by higher cytotoxic T cell and macrophage infiltration[23]. However, such features have not been shown to predict response to immune checkpoint inhibitors.

### *Common genomic alterations*

Molecular characteristics of mesothelioma have been found to influence overall prognosis and have been recently proposed to be integrated with histopathological reporting. Common among them are *CDKN2A* (p16) deletion (associated with poorer prognosis), BAP1 loss (associated with favorable prognosis), *LATS2* mutation (associated with poorer prognosis), and YAP1 overexpression[24]. Other genetic biomarkers like p53, NF2, W1F1, and DNA methylation have been associated with predicting prognosis but have limited clinical implications[25,26]. In a recent study, BAP1 loss by IHC has been demonstrated to predict superior overall survival following first-line platinum-pemetrexed com-bination.

### *Staging of mesothelioma*

The rarity of the tumor with finite prospective data has limited the development of accurate staging systems. However, using prospective data from over 3700 cases, the International Association for The Study of Lung Cancer has updated their Tumor-Node-Metastasis ($8^{th}$) staging for pleural mesothelioma[27]. Involvement of ipsilateral parietal or visceral pleural has been included in T1, whereas invasion into diaphragmatic muscle or lung has been categorized as T2. Involvement of chest wall soft tissue (solitary focus), mediastinal fat, or non-transmural pericardial involvement has been classified as T3. T1, T2, and T3 tumors are considered as potentially resectable. Pericardial involvement with or without effusion is classified as T4, which also includes involvement of contralateral pleura and peritoneal involvement. Involvement of any ipsilateral regional lymph node (intrathoracic, internal mammary, or scalene nodes) is classified as N1, whereas contralateral involvement or any-side supraclavicular involvement is classified as N2. The metastasis descriptor has been left unchanged[28].

## TREATMENT

MPM is insidious at the onset and slowly progressive. The patient usually presents with extensive intrathoracic disease. The prognosis is poor with a median survival of 10 months to 22 months. The optimal treatment is still not established;

however, early diagnosis with a multimodal treatment approach is imperative to improve outcomes in MPM. The treatment strategies include surgery, radiation therapy, chemotherapy, targeted treatment, and immunotherapy. Treatment depends on the extent of the disease, histological subtype, age, comorbidities, and performance status. Palliative treatment plays a crucial role in management, and it should be incorporated in the practice right from the diagnosis as patients with MPM have a significant symptom burden and palliative care needs, which if addressed can improve the quality of life. Figure 1 illustrates the general approach towards the management of MPM, encompassing diagnostic procedures, therapeutic strategies, and multidisciplinary care pathways.

## SURGERY

The goal of surgery in MPM is macroscopic complete resection. It should be considered in selected patients with disease limited to one hemithorax, appropriate cardiopulmonary reserve, good performance status, epithelioid or biphasic histology, and if complete cytoreduction can be achieved. It should be performed in specialized centers where appropriate expertise is available. As there are no randomized trials indicating survival benefit with surgery, the role of surgery remains uncertain. The main surgical approaches are extrapleural pneumonectomy (EPP), extended pleurectomy/decortication, and pleurectomy/decortication (P/D). The choice of surgical approach (EPP *vs* P/D) depends on expertise or preferences of the surgeon, the patient's medical condition, and availability of multimodal treatment strategies within that center.

The more radical EPP approach consists of en bloc resection of the ipsilateral lung with parietal and visceral pleura, pericardium, and diaphragm. The pericardium and diaphragm may be left intact if they are not involved with the tumor. In extended pleurectomy/decortication, parietal and visceral pleurectomy is performed with resection of the pericardium and diaphragm. The other less radical approach, P/D, consists of removal of parietal and visceral pleura without resection of the pericardium or diaphragm[29].

There are no randomized trials that compare these surgical approaches. A recently published meta-analysis of EPP *vs* P/D, which included 18 studies with total of 4852 patients, showed better median survival and reduced 30-day mortality and complications in the P/D group[30]. The morbidity and mortality were higher after EPP leading to adoption of P/D by various thoracic surgeons[31,32]. The common complications following EPP are cardiac arrhythmias, pulmonary embolism, pneumonia, broncho-pleural fistula, and respiratory failure. The most common complication after P/D is prolonged air leak[33].

The Mesothelioma and Radical Surgery trial evaluated the efficacy of EPP in the context of trimodality therapy. All patients received three initial cycles of platinum-based chemotherapy followed by clinical assessment. Patients were then randomized (1:1) to EPP ($n$ = 24) followed by postoperative hemithoracic radiotherapy or to no EPP ($n$ = 26). The trimodality therapy did not result in any survival benefit but was associated with high morbidity[34]. In contrast to the Mesothelioma and Radical Surgery trial, the Surgery for Mesothelioma After Radiotherapy (SMART) trial[35] demonstrated the benefit of surgical resection using EPP in resectable patients with clinical stage T1-3N0M0. The treatment protocol consisted of a short course of accelerated hemithoracic radiotherapy of 25 Gy in five fractions over 1 week followed by extrapleural pneumonectomy followed by adjuvant chemotherapy in patients with pathological mediastinal lymph node involvement. The target volume includes the entire hemithorax including diaphragmatic attachments, ipsilateral mediastinal and upper retroperitoneal nodes, and any chest tube or biopsy tracts. The entire irradiated lung was removed, thus reducing the radiation pneumonitis risk. Patients with ypN0 epithelial histology had better overall survival and disease-free survival. The SMART trial has shown that EPP after radiotherapy resulted in improvements in median overall survival but is associated with morbidity. The SMART protocol should be carried out in centers with surgical and radiation expertise.

## RADIOTHERAPY

A traditional treatment approach for pleural mesothelioma includes surgery and systemic therapy followed by radiotherapy as adjuvant treatment with radical or palliative intent. Recent advances in radiotherapy techniques have improved treatment accuracy and allows delivery of radical doses with fewer toxicities. The former perception is that mesothelioma is intrinsically radioresistant, but certain studies have shown that it responds better to a high dose per fraction. Various studies where radiotherapy has been used in palliative setting have shown better response with a high dose per fraction (4 Gy/# compared to 3 Gy/#). This is explained by a low $\alpha/\beta$ ratio, low proliferation index, and non-squamous histology[36].

The role of radiotherapy in pleural mesothelioma can be: (1) Hemithorax radiation prior to or after EPP; (2) Procedure tract radiation; (3) Hemithorax radiation after P/D; and (4) Palliation of local symptoms caused by disease[37].

For simulation for radiotherapy, after proper immobilization a free breathing or four-dimensional CT scan is recommended for patients undergoing hemithoracic radiation. Image guided target delineation using fluorodeoxyglucose PET has shown significant alterations in target volumes and thus improves treatment accuracy. MRI is also considered a preferred imaging modality for T3 or T4 disease for delineating pleural gross tumor volume[38].

Hemithoracic radiation is the standard of care post EPP for medically operable stage I to III patients, consistent with the National Comprehensive Cancer Center guidelines (version 2.2018) and American Society of Clinical Oncology 2018 recommendations. The radiotherapy technique has evolved over the years from conventional anteroposterior/posteroanterior fields to three-dimensional conformal radiotherapy to intensity modulated radiotherapy, which is currently the

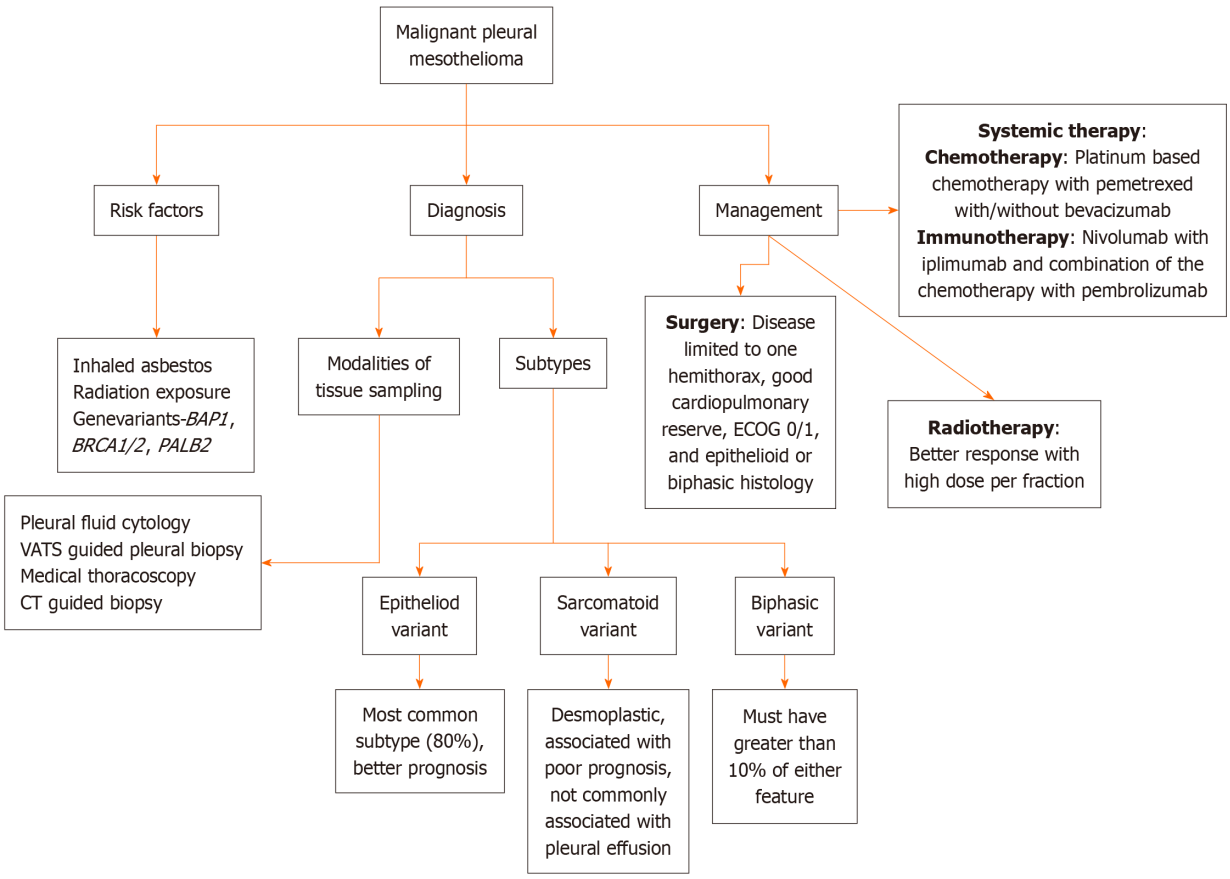


**Figure 1 General approach towards management of malignant pleural mesothelioma.** CT: Computed tomography; ECOG: Eastern Cooperative Oncology Group; VATS: Video-assisted thoracoscopic surgery.

standard of care. The main concern for patients undergoing intensity modulated radiotherapy for mesothelioma is the associated risk of pneumonitis due to large volumes of treatment after a pneumonectomy[39]. The recommended constraints after pneumonectomy is to keep the opposite lung V5 < 60%, V20 < 4 - 7%, and mean lung dose to < 8 Gy[39, 40]. The postoperative field encompasses the entire pleural bed. The clinical target volume includes ribs in the lateral aspect along with a margin at the pleural/mediastinal interface followed by a planning target volume expansion of 0.5 cm. A dose of 45-54 Gy in 1.8-2.0 Gy/ fraction and a boost to 54 to 60 Gy for R1 or R2 resection is recommended according to the International Association for The Study of Lung Cancer guidelines. Prophylactic irradiation of mediastinal nodes is not recommended.

With recent progress towards more conservative lung-sparing procedures and the availability of advanced radiation techniques, the use of EPP has declined. Multimodality approach using hemithoracic intensity-modulated pleural radiation therapy technique along with P/D and chemotherapy with a dose of 50.4 Gy in 28 fractions is feasible and could be delivered safely with no reported grade 4 or grade 5 pneumonitis[41]. Additional advanced modalities such as proton therapy are emerging. Stereotactic radiotherapy for recurrent pleural mesothelioma has been studied with promising results. Palliative radiotherapy is used for symptom control.

## SYSTEMIC TREATMENT

The standard systemic treatment for mesothelioma was a combination of pemetrexed and cisplatin with carboplatin being used if cisplatin was contraindicated[42,43]. In the phase 3 EMPHACIS trial by Vogelzang *et al*[42], a combination of pemetrexed/cisplatin chemotherapy (pemetrexed 500 mg/m$^2$ and cisplatin 75 mg/m$^2$ three times weekly) resulted in a response rate of 41.3% compared to 16.7% with cisplatin alone with a median overall survival of 12.1 months *vs* 9.3 months for cisplatin alone. Supplementation with folic acid and vitamin B12 resulted in a significant reduction in toxicities and greater improvement in all efficacy parameters in the pemetrexed/cisplatin chemotherapy arm. There were no adverse effects of supplements. Patients who received vitamin supplementation were able to receive more cycles of chemotherapy, which might have also contributed to the results. This trial established a combination of pemetrexed and cisplatin as a new standard of care in systemic treatment for mesothelioma. Since then, this combination of platinum and pemetrexed has been used as the backbone of combination therapies and the control arm in various trials.

The role of maintenance pemetrexed in mesothelioma is still not defined. In a phase 2 trial (CALGB 30901) of maintenance pemetrexed *vs* observation, patients with unresectable disease were randomized to maintenance pemetrexed after four to six cycles of pemetrexed and platinum. A total of 49 patients with 27 in the pemetrexed arm and 22 in the observation arm were included. The median overall survival was 11.8 months for the observation arm and 16.3 months for the maintenance arm ($P$ = 0.67). The progression free survival (PFS) was 3.4 months in the maintenance arm and 3 months in the observation arm ($P$ = 0.97). Thus, maintenance pemetrexed did not result in improvement of PFS and did not provide any additional benefit[44].

The addition of bevacizumab to the pemetrexed/cisplatin doublet was tested in the phase 3 Mesothelioma Avastin Cisplatin Pemetrexed Study trial[45]. Patients received pemetrexed/cisplatin combination or 15 mg/kg bevacizumab on day 1 in addition to pemetrexed/cisplatin combination (PCB) in three weekly cycles for up to six cycles. The median overall survival was significantly longer with PCB (18.8 months *vs* 16.1 months, $P$ = 0.0167). Similarly, PFS was better with PCB (median PFS 9.2 months *vs* doublet 7.3 months, $P$ < 0.0001). The grade 3-4 adverse effects were more in the PCB group resulting in more patients stopping the treatment, but there was no worsening of quality of life.

The United States Food and Drug Administration approved the use of tumor treating fields (TTF) in combination with systemic therapy with pemetrexed and platinum chemotherapy for first-line of treatment of unresectable mesothelioma following the publication of the STELLAR trial in 2019[46]. Patients were treated with continuous TTF (≥ 18 h a day with 150 kHz) to the thorax with concomitant chemotherapy with pemetrexed and platinum combination every 3 weeks for up to six cycles. TTF were given as maintenance treatment to patients whose disease did not progress following completion of chemotherapy. The combination of TTF and chemotherapy resulted in a median overall survival of 18.2 months.

Immunotherapy (IO) has restructured the therapeutic armamentarium in mesothelioma. The combination of nivolumab plus ipilimumab was approved as first-line systemic treatment for mesothelioma based on results of the CheckMate 743 trial[47]. In this multicentric randomized phase 3 study, patients were randomized to nivolumab 3 mg/kg every 2 weeks plus ipilimumab 1 mg/kg every 6 weeks for up to 2 years or platinum plus pemetrexed chemotherapy (cisplatin or carboplatin) once every 3 weeks for up to six cycles. The IO combination significantly improved median overall survival compared to chemotherapy (18.1 months *vs* 14.1 months, $P$ = 0.002). The 2-year overall survival was 41% in the dual IO combination *vs* 27% in the chemotherapy group. Due to toxicity, 15% of patients discontinued treatment in the dual IO group compared to 7% in the chemotherapy group. The median overall survival with dual IO was similar between epithelioid (18.7 months) and non-epithelioid types (18.1 months). However, the median overall survival with chemotherapy in non-epithelioid type was 8.8 months and 16.5 months in epithelioid histology. The magnitude of benefit with dual IO was thus greater in the non-epithelioid histology. On subgroup analysis based on PD-L1 expression, survival outcomes with dual IO were similar in the patients with less than 1% and with 1% or higher PD-L1 expression. The 2-year outcomes were better with nivolumab plus ipilimumab than with chemotherapy in both the subgroups. The updated results of CheckMate 743[48] with a median follow-up of 43 months published in 2022 showed that the combination of nivolumab and ipilimumab continued to prolong overall survival over chemotherapy with a tolerable side effect profile. Twenty-eight percent of the patients continued to have an ongoing response at 3 years.

Based on results of this trial, nivolumab and ipilimumab became the standard of care for patients with non-epithelioid pleural mesothelioma. For patients with epithelioid histology, chemotherapy remains the treatment of choice, and immunotherapy can be kept as an alternative.

The combination of pembrolizumab with pemetrexed/platinum was tested against pemetrexed/platinum in a phase 3 randomized trial conducted at 51 hospitals[49]. Chemotherapy was given in standard doses with or without intravenous pembrolizumab 200 mg every 3 weeks for up to 2 years. Median survival was 17.3 months with pembrolizumab compared to 16.1 months with chemotherapy alone ($P$ = 0·0324). The combination of pembrolizumab with chemotherapy improved the survival and was tolerable.

There are currently no approved second-line treatment options for patients progressing on first-line treatment. The treatment approach depends on the initial regimen used for first-line treatment. Patients treated initially with IO can be treated with chemotherapy with pemetrexed/platinum combination or singlet depending on performance status. For patients who have been treated initially with chemotherapy, the second-line treatment depends on the treatment-free period after the first-line chemotherapy. The options available are re-challenging patients with the initial regimen if the gap is ≥ 6 months or IO or single agent chemotherapy.

## CONCLUSION

MPM is a rare and aggressive malignancy with a dismal prognosis. Management of MPM is challenging and requires a multimodal and holistic approach. Before the positive trial of dual IO, chemotherapy with pemetrexed and platinum has been the standard of care for nearly two decades. There has been recent significant progress in the management with nivolumab and ipilimumab becoming the new standard of care for non-epithelioid type mesothelioma. Palliative care is an integral component of the treatment paradigm to reduce the symptom burden and maintain or improve quality of life. There is a need of translational biomarker studies to identify the patients who will benefit from IO or chemotherapy and bridge the gap between the current scenario and desired future. There is still a long road ahead to improve the overall management of MPM.

## FOOTNOTES

**Author contributions:** Khosla D and Singh PK contributed to conceptualization, manuscript preparation, writing, and editing and were responsible for the integrity of article; Chhabria BA and Kataria V contributed to literature search, manuscript writing, and editing; Singh N and Kapoor R reviewed and approved the manuscript.

**Conflict-of-interest statement:** All authors declare no conflicts of interest.



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**S-Editor:** Liu JH
**L-Editor:** Filipodia
**P-Editor:** Wang WB

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*World J Exp Med* 2024 September 20; 14(3): 96042

DOI: 10.5493/wjem.v14.i3.96042

ISSN 2220-315x (online)

*OPINION REVIEW*

# Artificial intelligence as a tool in drug discovery and development

Maria Kokudeva, Mincho Vichev, Emilia Naseva, Dimitrina Georgieva Miteva, Tsvetelina Velikova

**Specialty type:** Research and experimental medicine

**Provenance and peer review:** Invited article; Externally peer reviewed.

**Peer-review model:** Single blind

**Peer-review report's classification**

**Scientific Quality:** Grade B
**Novelty:** Grade B
**Creativity or Innovation:** Grade C
**Scientific Significance:** Grade B

**P-Reviewer:** Ye XJ

**Received:** April 25, 2024
**Revised:** August 6, 2024
**Accepted:** August 12, 2024
**Published online:** September 20, 2024
**Processing time:** 125 Days and 18.4 Hours

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## Abstract

The rapidly advancing field of artificial intelligence (AI) has garnered substantial attention for its potential application in drug discovery and development. This opinion review critically examined the feasibility and prospects of integrating AI as a transformative tool in the pharmaceutical industry. AI, encompassing machine learning algorithms, deep learning, and data analytics, offers unprecedented opportunities to streamline and enhance various stages of drug development. This opinion review delved into the current landscape of AI-driven approaches, discussing their utilization in target identification, lead optimization, and predictive modeling of pharmacokinetics and toxicity. We aimed to scrutinize the integration of large-scale omics data, electronic health records, and chemical informatics, highlighting the power of AI in uncovering novel therapeutic targets and accelerating drug repurposing strategies. Despite the considerable potential of AI, the review also addressed inherent challenges, including data privacy concerns, interpretability of AI models, and the need for robust validation in real-world clinical settings. Additionally, we explored ethical considerations surrounding AI-driven decision-making in drug development. This opinion review provided a nuanced perspective on the transformative role of AI in drug discovery by discussing the existing literature and emerging trends, presenting critical insights and addressing potential hurdles. In conclusion, this study aimed to stimulate discourse within the scientific community and guide future endeavors to harness the full potential of AI in drug development.

**Key Words:** Artificial intelligence; Drug discovery; Drug development; Decision-making; AI-driven medicine; Healthcare; Public health



**Core Tip:** Embracing artificial intelligence (AI) expedites drug discovery and development by streamlining computational chemistry, molecular modeling, and data mining. Leveraging AI-driven algorithms enhances the accuracy and efficiency of identifying potential drug candidates and predicting their pharmacological properties. Integrating machine learning and deep learning frameworks into pharmaceutical research optimizes decision-making, accelerates drug design cycles, and ultimately advances novel therapies for various diseases.

**Citation:** Kokudeva M, Vichev M, Naseva E, Miteva DG, Velikova T. Artificial intelligence as a tool in drug discovery and development. *World J Exp Med* 2024; 14(3): 96042
**URL:** https://www.wjgnet.com/2220-315x/full/v14/i3/96042.htm
**DOI:** https://dx.doi.org/10.5493/wjem.v14.i3.96042

## INTRODUCTION

Drug discovery and development is a long, expensive, and complex process that can often take more than 10 years from molecule identification to medical drug approval and placement on the market. Each stage in the process carries a risk of failure, and most drug applicants never reach the market. This makes the process of drug innovation and development both expensive and inefficient[1,2].

In recent years, the use of artificial intelligence (AI) in this industry has increased significantly. Drug discovery requires the analysis of large databases of chemical compounds. This can be achieved rapidly using machine learning techniques [3]. These techniques have their limitations because even a little change in the molecular structure of the drug can drastically alter its effect. Drug discovery involves the analysis and comparison of the properties of different molecular structures and components. In this context, AI tools can automatically scan large datasets quickly, using a composition safety check to pick out the most effective model for a certain goal[4,5].

Several public libraries store chemical and biological data, including ChEMBL[6] and PubChem[7]. They contain information on millions of molecules for various disease targets. These libraries are machine-readable and are used for drug discovery models, including for drug candidate compounds targeting severe acute respiratory syndrome coronavirus 2[8]. AI (mostly machine learning techniques) has also been implemented to evaluate toxicity. For example, the DeepTox platform is used as a model to evaluate the toxicity of certain compounds[9]. Another platform, MoleculeNet, can be used to translate molecular structures and predict toxicity[10].

The assessment of drug-target interactions is another important stage of drug design. The binding affinity between the drug and its target is important for the final product. Molecular docking, one of the most common approaches to predict affinity, is used to study the binding and complex formation between two molecules, such as receptor-ligand interactions [11,12].

Different pharmaceutical companies have used AI to improve drug discovery. Verge Genomics uses AI to predict the effects of some new drugs on patients with Alzheimer's disease and Parkinson's disease[13]. Despite their use of automated data analysis, certain drug studies have failed. Most neurological diseases are polygenic, but the company drug data targets one gene. In 2018, Verge Genomics developed an algorithm to identify the pathogenic genes and select drugs to target them all. Thus, the company successfully utilized the vast potential of AI and machine learning algorithms by identifying drugs for neurodegenerative diseases. In 2018, Bayer and Merck received Food and Drug Administration approval to use AI algorithms to support clinical decision making for chronic thromboembolic pulmonary hypertension [14]. This form of chronic thromboembolic pulmonary hypertension is very rare and affects approximately 5/1000000 people annually worldwide. The symptoms resemble chronic obstructive pulmonary disease or asthma, complicating its diagnosis even after numerous medical tests.

Novartis currently uses AI algorithms to classify digital images of different cells[15,16]. Each cell is treated with different experimental molecules. The algorithms group and test molecules with similar effects. Finding biologically active molecules requires complicated analysis. Therefore, to speed up this screening process, Novartis research teams use machine learning algorithms to predict which unknown molecules might be worth exploring.

In 2018, the biotech company Cyclica collaborated with Bayer[17], using AI machine learning to determine the polypharmacological profiles of small molecules and develop more affordable drugs. The company created Ligand Express, an integrated network of cloud technologies expanded with AI that enhances drug design, screening, and personalization.

In line with those mentioned above, AI-driven drug design, development, and delivery are highly trendy topics to discuss. We hypothesize that integrating AI into drug discovery and development processes will significantly enhance efficiency, accuracy, and innovation, leading to the discovery of novel therapeutic agents and the optimization of existing drugs. Our goals for this review were: (1) To assess the current state of AI applications in drug discovery, including

machine learning algorithms, predictive modeling, and virtual screening techniques; (2) To explore recent advancements in AI-driven platforms and technologies that have revolutionized drug discovery, such as deep learning algorithms, generative models, and molecular design tools; (3) To investigate how AI has impacted various stages of drug development, from target identification and lead optimization to clinical trials and post-marketing surveillance; (4) To showcase case studies and success stories where AI-driven approaches have led to the discovery of promising drug candidates or repurposing of existing drugs for new indications; (5) To identify key challenges and limitations associated with AI in drug discovery, such as data quality issues, ethical considerations, and regulatory hurdles; and (6) To provide insights into potential future directions and emerging trends in AI-enabled drug discovery, including the integration of multi-omics data, collaborative AI platforms, and AI-driven personalized medicine approaches. The current review is crucial as it addresses the intersection of two rapidly evolving fields: AI and drug discovery. Our review aimed to provide a comprehensive summary for AI experts, drug developers, and healthcare professionals by synthesizing the latest research, methodologies, and best practices. The insights gained from this review will inform academia and industry and contribute to the ongoing efforts to accelerate drug discovery, improve patient outcomes, and address unmet medical needs globally.

## SEARCH STRATEGY

We conducted a comprehensive search across multiple databases, including PubMed, Scopus, Web of Science, IEEE Xplore, and Google Scholar. We focused on various types of studies, such as review articles, research articles, case studies, clinical trials, and meta-analyses. Using Boolean operators, our search terms included ("Artificial intelligence" OR "AI") AND ("Drug discovery" OR "Drug development") AND ("Machine learning" OR "Deep learning" OR "Neural networks") AND ("Pharmaceuticals" OR "Medications" OR "Compounds"), ("Computational chemistry" OR "Chemoinformatics") AND ("Drug design" OR "Molecular modeling"), and ("Data mining" OR "Big data analytics") AND ("Pharmacology" OR "Therapeutics"). This strategy allowed us to gather relevant literature on the application of AI in various stages of drug discovery and development, including computational chemistry, molecular modeling, data mining, and pharmacological analysis. The paper selection is presented in Figure 1.

## CURRENT LANDSCAPE OF DRUG DISCOVERY

### *Traditional methods and limitations*

Historically, drug discovery has relied heavily on trial-and-error methods, where researchers would test compounds to see if they had the desired therapeutic effect[18]. However, traditional methods are associated with high failure rates and lengthy timelines. It often takes years and significant resources to develop a single drug, with many candidates failing in clinical trials[19]. Moreover, traditional methods often lack the depth of understanding required to fully grasp complex biological systems and disease mechanisms[20].

### *Evolution of technology in pharmaceutical research*

High-throughput screening techniques allow researchers to quickly screen large libraries of compounds for potential drug candidates, speeding up the initial stages of drug discovery[21]. Advances in genomics, proteomics, and other omics technologies have provided researchers with a deeper understanding of biological systems and disease pathways[22]. Computational approaches have become increasingly important, allowing researchers to simulate molecular interactions and predict the properties of potential drug candidates[23].

### *Emergence of AI in drug discovery*

AI techniques, particularly machine learning and deep learning, have revolutionized drug discovery by analyzing large datasets, predicting molecular properties, and identifying potential drug candidates[24]. AI algorithms can perform virtual screening of compound libraries to identify molecules with the highest likelihood of binding to specific targets, reducing the time and cost associated with experimental screening[25]. AI models can predict the pharmacokinetic and pharmacodynamic properties of compounds, helping researchers prioritize the most promising candidates for further development[26].

AI algorithms can analyze patient data to identify biomarkers, predict treatment responses, and tailor therapies to individual patients, leading to the development of more effective and targeted treatments[27]. The integration of AI into drug discovery and development has the potential to significantly accelerate the process, reduce costs, and improve the success rate of bringing new drugs to market. However, challenges, such as data quality, regulatory considerations, and ethical concerns, must be addressed to fully realize the benefits of AI in pharmaceutical research.

## APPLICATIONS OF AI IN DRUG DISCOVERY

### *Target identification and validation*

The process of drug discovery starts with molecule searching. Certain small molecule databases can match specific health

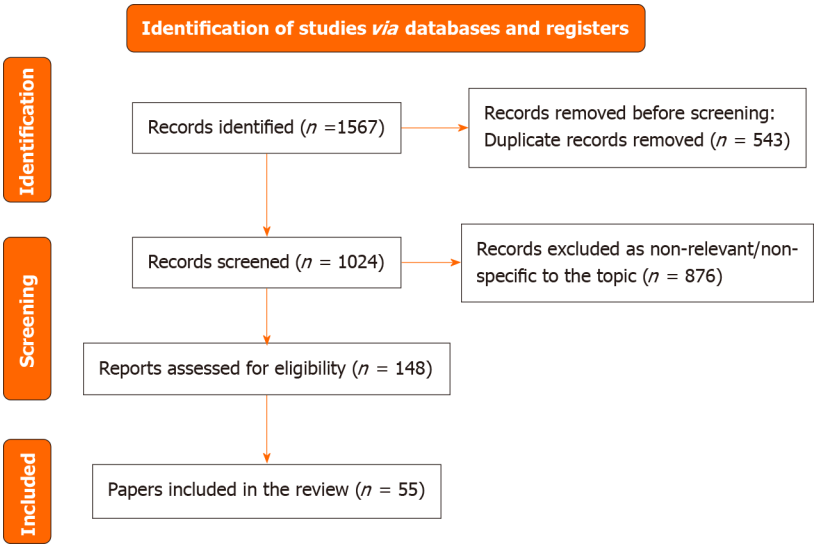


**Figure 1 Identification, screening, and selection of papers to include.**

problems. Ranges of docking software are developed to explore molecular bioactivity[28]. Researchers also use analogues of already known molecules[29].

### *High-throughput screening and data analysis*

High-throughput screening is a process for identifying potential molecules to be included in further analysis. The molecules identified in the previous step are screened to identify the most applicable to the health problem of interest. The process also includes molecule stability and interactions[30]. Wide varieties of methods are employed, including neural network, multiple linear regression, decision tree, and the analysis of variance[31,32].

### *Predictive modeling for drug design*

Drug design is an important process. It includes the form (for example, tablet or solution) as well as the excipients (the other ingredients). AI is also applied at this stage. The tested parameters include the blend bulk and tapped density, flowability, angle of repose, appearance, friability, resistance to crushing, and time of disintegration of the tablet[33].

### *Biomarker discovery and validation*

In the era of molecular medicine, biomarker discovery enhances the drug discovery process. Biomarker discovery requires a large number of samples to be collected and thoroughly analyzed in a uniform manner. Validation ensures that the marker is reproducible and reliable, and that its sensitivity and specificity are acceptable. AI could be employed in this step. Within the drug development context, biomarkers are used as an outcome measure in clinical trials, helping the identification and validation of drug targets. Thus, the right treatment for each patient based on the biomarkers tested would be found[34].

## AI-DRIVEN DRUG DEVELOPMENT

In recent years, AI technology has revolutionized pharmaceutical research, ushering in a new era of drug development and revolutionizing medicine discovery, testing, and patient delivery. This revolution can transform various stages of the drug development pipeline, from accelerating preclinical research to optimizing clinical trial design and enabling personalized medicine approaches.

### *Accelerating preclinical research*

Preclinical research involves extensive and time-consuming safety and efficacy assessments of potential drug candidates. This process is expensive, challenging, and often unsuccessful. Here, AI can streamline data analysis, predict drug interactions, and identify promising compounds. Machine learning algorithms have been developed to predict the toxicity of potential drug candidates[35]. Using AI-driven platforms, researchers can rapidly screen thousands of compounds and prioritize the most promising candidates, significantly reducing the time and cost of preclinical testing [36].

### *Optimization of clinical trial design*

Conventional trial designs are often flawed and inefficient, leading to high costs, lengthy timelines, and sometimes inconclusive results. AI can improve patient recruitment, trial outcomes, and novel therapy development by tailoring inclusion criteria and treatment protocols based on predictive analytics[37]. In addition, AI-powered algorithms can

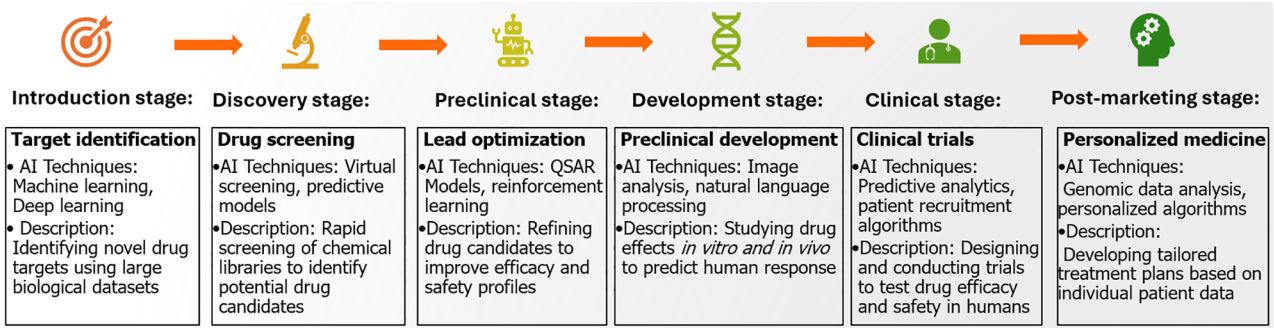


**Figure 2 An entire workflow of artificial intelligence applications in drug discovery and development, highlighting the different stages and the corresponding artificial intelligence techniques used.** AI: Artificial intelligence; QSAR: Quantitative structure-activity relationship. The figure was generated using brainstorming from OpenAI. (2024). *ChatGPT* [Large language model]. https://chatgpt.com/c/d95bc2d2-a53a-492d-a78d-6946bc43cef2.

analyze patient data, identify relevant biomarkers, and stratify patient populations to optimize trial design[38]. AI-driven simulations allow researchers to explore virtual trial scenarios, refine study protocols, and mitigate risks before initiating costly and time-consuming clinical trials.

### *Personalized medicine approaches*

The advancement of personalized medicine is one of the most promising applications of AI in drug development. The paradigm of disease treatment and drug development is shifting towards personalized therapies to achieve better results for individual patients. AI can accelerate this trend by improving diagnostics, collecting personalized information, and assisting clinical decisions[39]. AI algorithms can store and analyze patient data, such as genetic profiles, clinical histories, and lifestyle factors[40]. This can significantly alleviate the burden of extensive data collection and analysis on researchers, facilitating their speedy and efficient work by allowing them to focus on the clinical scenario. In addition, AI technology can identify biomarkers associated with drug responses or disease progression, further improving targeted therapies with maximal efficacy and minimal adverse effects[41]. In conclusion, AI-driven drug development can transform the pharmaceutical industry by optimizing preclinical research, clinical trial design, and personalized treatment. Researchers can benefit from AI to expedite the discovery and delivery of innovative therapies that address unmet clinical needs and improve patient outcomes.

We present an overview of AI utilization in drug development in Figure 2.

## CHALLENGES AND ETHICAL CONSIDERATIONS

### *Data quality and bias in AI models*

Ensuring the quality and reliability of data used to train AI models is crucial for accurate predictions and decision-making. Biases, inaccuracies, and incompleteness in the data can lead to flawed results[42]. AI models can inherit biases present in the training data, leading to biased predictions or decisions. This is particularly concerning in healthcare, where biases related to race, sex, or socioeconomic status can impact patient outcomes[43]. Developing techniques to identify and mitigate biases in AI models, as well as ensuring diverse and representative training datasets, are essential for ethical AI applications in drug discovery[44].

### *Interpretability and transparency*

Many AI models, particularly deep learning models, are often considered "black boxes" because their internal workings are not easily interpretable by humans. This lack of transparency raises concerns about how decisions are made and undermines trust in AI systems[45]. There is a growing need for interpretable AI models, where the reasoning behind predictions or recommendations can be understood by domain experts and regulatory authorities[46]. Establishing mechanisms to ensure accountability for AI-driven decisions, including transparency about model training and validation, is crucial for maintaining ethical standards in drug discovery[47].

### *Regulatory and ethical implications*

AI applications in drug discovery must adhere to regulatory requirements set forth by agencies, such as the Food and Drug Administration and European Medicines Agency. Ensuring compliance with regulations designed for traditional drug development processes presents challenges due to the unique nature of AI technologies[48]. Demonstrating the safety and efficacy of AI-generated drug candidates or treatment recommendations is essential for regulatory approval. Robust validation and testing procedures are necessary to mitigate risks to patients[49]. Protecting patient privacy and securing sensitive healthcare data used in AI applications is paramount. Adhering to data protection regulations, such as the General Data Protection Regulation and the Health Insurance Portability and Accountability Act, is essential to maintain trust and ethical standards[50].

**Table 1 Applications of artificial intelligence in drug discovery and development**

| Application area | AI techniques used | Key benefits | Challenges |
|---|---|---|---|
| Target identification | Machine learning, deep learning | Identifying novel drug targets, high accuracy | Data quality, complexity of biological systems |
| Drug screening | Virtual screening, predictive models | Faster screening of compounds, cost-effective | False positives/negatives, model validation |
| Lead optimization | QSAR models, reinforcement learning | Improved candidate selection, reduced development time | Integration with traditional methods, data scarcity |
| Preclinical development | Image analysis, natural language processing | Enhanced understanding of drug toxicity and efficacy | Interpretation of complex data, standardization |
| Clinical trials | Predictive analytics, patient recruitment algorithms | Optimized trial design, better patient stratification | Ethical concerns, data privacy |
| Personalized medicine | Genomic data analysis, personalized algorithms | Tailored treatments, improved patient outcomes | Data integration, regulatory issues |

AI: Artificial intelligence; QSAR: Quantitative structure-activity relationship.

Addressing these challenges and ethical considerations is crucial for the responsible and ethical use of AI in drug discovery and development. Collaborative efforts between researchers, regulatory bodies, and ethicists are essential to develop guidelines and frameworks that promote the ethical use of AI while maximizing its potential benefits in healthcare.

## FUTURE DIRECTIONS AND INNOVATIONS

### *Advancements in AI technologies*

Deep learning is advanced machine learning that could be applied in the field of drug discovery. It is a neural network that can extract information from public databases and create scientific conclusions based on them. Deep learning is applicable to reduce the costs of the clinical trials by predicting their outcome before they start[51]. Another promising application of AI in the field of drug discovery is drug repurposing. Finding new applications for already existing drugs reduces the time and cost of their development[52]. Another new trend in the field of drug discovery and development is AI application in nanotechnologies, especially nanocarriers. AI is also crucial in smart drug release systems that deliver the medicine when it is needed[53].

### *Collaborative approaches and industry trends*

Application of AI in the field of drug discovery requires a multidisciplinary approach by default. Collaboration between researchers, clinical experts, engineers, and data managers is crucial. Thus, multidisciplinary education is required to meet the new demands of pharmaceutical trends[53].

### *Potential impact on drug development pipelines*

AI can speed up the drug discovery and development process and reduce costs. The resources not allocated to drug discovery could be invested into drug searching for different diseases. This could have a large positive impact on public health.

## INTEGRATION OF AI INTO MAINSTREAM DRUG DISCOVERY

The use of AI in mainstream drug research signifies a watershed moment set to transform the pharmaceutical business. Adoption strategies and industry readiness are critical components of this transformation, which need strong frameworks for AI adoption and organizational readiness[54].

Training and skill development programs are critical for providing professionals with the requisite skills in AI-driven approaches, guaranteeing smooth integration and realizing the potential advantages. Overcoming industrial opposition and skepticism requires proactive actions to address concerns about AI technology dependability, ethical issues, and data security. Collaboration among stakeholders, regulatory authorities, and AI developers is critical for building confidence and accelerating wider adoption[54]. As AI evolves and demonstrates its usefulness in drug development, proactive involvement, ongoing education, and open communication will be critical in managing difficulties and maximizing the promise of AI-driven advances in the pharmaceutical sector.

In Table 1, we present the AI techniques used for drug development, their application areas, key benefits, and challenges.

## CONSIDERATIONS FOR SMALL AND LARGE PHARMACEUTICAL COMPANIES

### *Customization of AI tools*

Small pharmaceutical companies may benefit from customizable AI tools that are tailored to their specific research needs and capabilities. Customization allows them to focus on targeted areas of drug discovery where AI can provide the most value. Larger pharmaceutical companies may have the resources to develop or acquire sophisticated AI platforms that can be customized for various stages of drug development, from target identification to clinical trial optimization. Customization enables them to integrate AI seamlessly into their existing workflow and infrastructure[55].

### *Resource allocation and return on investment*

Small pharmaceutical companies must carefully allocate limited resources when investing in AI technologies. They need to assess the potential return on investment of implementing AI tools and prioritize projects with the highest likelihood of success. Large pharmaceutical companies have greater financial resources and may allocate significant budgets to AI initiatives[55]. However, they also face pressure to demonstrate tangible return on investment and ensure that AI investments align with strategic business objectives.

## GLOBAL COLLABORATIONS AND DATA SHARING

Small pharmaceutical companies may lack access to large datasets necessary to train AI models effectively. Collaborating with academic institutions, research organizations, or larger pharmaceutical companies can provide access to diverse datasets and expertise, facilitating more robust AI applications. In contrast, large pharmaceutical companies often have extensive internal datasets, but collaboration with external partners can still be beneficial. Engaging in global collaborations and data sharing initiatives allows them to access additional resources, validate AI models across diverse populations, and accelerate drug discovery efforts[53].

Both small and large pharmaceutical companies can leverage AI in drug discovery and development, but considerations, such as customization of AI tools, resource allocation, and global collaborations differ based on their size, resources, and organizational capabilities.

## CONCLUSION

This review showed that integrating AI can revolutionize drug discovery and development. Utilizing machine learning algorithms, deep learning techniques, and data analytics, AI can expedite target identification, optimize lead compounds, and predict pharmacokinetics and toxicity. By acknowledging the inherent challenges, including insufficient resources and model interpretability on a larger scale, this review demonstrated the need for robust validation and ethical considerations in AI-driven drug development. Nevertheless, the future demonstrates a pressing need for industry stakeholder and research community collaboration to overcome these hurdles and harness the full potential of AI to drive innovation and improve patient outcomes in medical research.

## FOOTNOTES

**Author contributions:** Kokudeva M and Vichev M were involved equally in conceptualizing the idea and writing the draft; Miteva D, Naseva E and Velikova T wrote additional sections in the paper; Vichev M was responsible for the critical revision of the manuscript for relevant intellectual content; Velikova T was responsible for project administration and funding acquisition; All authors approved the final version of the paper prior to submission.

**Supported by** the European Union-NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, No. BG-RRP-2.004-0008.

**Conflict-of-interest statement:** All authors declare they have no conflicts of interest to disclose.



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**S-Editor:** Liu JH
**L-Editor:** Filipodia

**P-Editor:** Yu HG

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World Journal of Experimental Medicine

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*World J Exp Med* 2024 September 20; 14(3): 98525

DOI: 10.5493/wjem.v14.i3.98525

ISSN 2220-315x (online)

*REVIEW*

# Anal carcinoma - exploring the epidemiology, risk factors, pathophysiology, diagnosis, and treatment

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**Specialty type:** Medicine, research and experimental

**Provenance and peer review:** Invited article; Externally peer reviewed.

**Peer-review model:** Single blind

**Peer-review report's classification**
**Scientific Quality:** Grade C
**Novelty:** Grade C
**Creativity or Innovation:** Grade C
**Scientific Significance:** Grade B

**P-Reviewer:** Ding MX

**Received:** June 28, 2024
**Revised:** July 15, 2024
**Accepted:** August 6, 2024
**Published online:** September 20, 2024
**Processing time:** 61 Days and 23.9 Hours

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## Abstract

Anal carcinoma is a relatively rare tumor that accounts for approximately 2% of gastrointestinal malignancies and less than 7% of anorectal cancers. Most anal tumors originate between the anorectal junction and the anal verge. Risk factors for the disease include human papillomavirus infection, human immunodeficiency virus, tobacco use, immunosuppression, female sex, and older age. The pathogenesis of anal carcinoma is believed to be linked to human papillomavirus-related inflammation, leading to dysplasia and progression to cancer. Squamous cell carcinoma is the most common type of anal tumor, with an annual incidence of approximately 1 to 2 per 100000 persons. Treatment regarding anal cancer has emerged over time. However, chemoradiation therapy remains the mainstay approach for early localized disease. Patients with metastatic disease are treated with systemic therapy, and salvage surgery is reserved for disease recurrence following chemoradiation. This article aims to provide background information on the epidemiology, risk factors, pathology, diagnosis, and current trends in the management of anal cancer. Future directions are briefly discussed.

**Key Words:** Anal cancer; Squamous cell carcinoma; Chemoradiation therapy; Human papillomavirus; Immunosuppression

**Core Tip:** Anal cancer is a rare malignancy, comprising 1%-6% of anorectal tumors. The incidence of anal carcinoma has been steadily increasing worldwide, and diagnosis is often challenging due to the similarities in clinical presentation to benign rectal diseases such as hemorrhoids. Roughly 85% of anal cancers are squamous cell carcinoma, and human papillomavirus infection and immunosuppression are major risk factors for the disease. Chemoradiation is the treatment of choice for early-stage cancer, while systemic therapy is used for metastatic disease. Novel cytotoxic agents in combination with immunotherapy have produced favorable outcomes in patients with advanced disease.

**Citation:** English KJ. Anal carcinoma - exploring the epidemiology, risk factors, pathophysiology, diagnosis, and treatment. *World J Exp Med* 2024; 14(3): 98525
**URL:** https://www.wjgnet.com/2220-315x/full/v14/i3/98525.htm
**DOI:** https://dx.doi.org/10.5493/wjem.v14.i3.98525

## INTRODUCTION

Anal carcinoma is a rare disease entity that accounts for less than 5% of cancers of the intestine[1]. In the general population, the incidence of anal cancer has steadily increased from 1.2 in 1992 up to 1.9 per 100000 persons in the United States alone, according to data from the National Cancer Institute's Surveillance, Epidemiology, and End Results Program in 2024[2]. Among anal cancers, squamous cell carcinoma (SCC) is the predominant histological subtype, followed by adenocarcinoma and rarer tumors such as basal cell carcinoma (BCC), melanoma, and small cell carcinoma[3,4].

Treatment of anal tumors has evolved over several decades, with concurrent mechanism identification. The primary choice of management has undergone a radical shift from abdominoperineal resection (APR) to organ-preserving chemoradiation therapy (CRT) and surgery[5]. Furthermore, recent developments using immunotherapy for advanced disease and metastasectomy in selected cases highlight the continuous changes and evolution of disease management[6,7]. In this article, we provide a scoping review of anal carcinoma and its epidemiology, risk factors, pathophysiology, diagnosis, and management.

## EPIDEMIOLOGY

Anal cancer is an uncommon tumor that comprises less than 2% of large bowel malignancies and approximately 1%-6% of anorectal tumors[1,3,4]. This cancer has become more prevalent in Western nations such as Australia and the United States, with little to no change in Asian countries and Spain[8]. In the United States, between the period of 1994 to 2000, the incidence of anal cancer almost doubled, with a rate of 2.04 per 100000 in males and 2.06 per 100000 in females, in comparison with the period between 1973 and 1979, where the incidence was 1.06 per 100000 in males and 1.39 per 100000 in females[2,5]. Incidence rates in the United Kingdom and across Europe range from 0.7 to 1.7 per 100000 persons per year[9,10].

In 2020, the global anal cancer incidence was estimated at 0.65 per 100000 persons, with 50865 cases[11]. The worldwide incidence of anal SCC was 8.2, with a lower incidence observed in the male population (0.28 *vs* 0.53 in females) and a higher proportion observed in Northern and Western Europe, North America, and Oceania[11,12]. The global incidence of anal adenocarcinoma was 0.54 in the same year, with a higher incidence observed in the male population (0.25 *vs* 0.19 in females) and a higher proportion observed in eastern Asia and northern and sub-Saharan Africa[11,13]. The growing trend of anal cancer is believed to be linked to the rising rates of human papillomavirus (HPV) infection.

## RISK FACTORS

The development of anal cancer is linked to multiple risk factors. Social and cultural dynamics over the last few decades have played a significant role in patient exposure and increased rates of anal tumors. The most notable risk factors are as follows.

### *HPV infection*

HPVs are a group of double-stranded circular DNA oncovirus from the Papillomavirdae family, commonly transmitted by mucosa-mucosa or skin-to-skin contact and entering the body by mucosal or cutaneous trauma in the form of oral sex, vaginal, or anal intercourse[14]. The infection is the most common sexually transmitted disease worldwide, with prevalence variation regionally[15]. The lifetime risk of infection at least once among men and women is 50%, and HPV serves as the underlying cause of cervical cancer[14,16,17]. In fact, female patients with cervical cancers are at increased risk for anal cancer[18].

Anal malignancies are often associated with HPV infection, although it is relatively uncommon for most patients with this infection to acquire anal cancer[12,19]. Anal carcinoma is commonly linked to HPV infection, most notably subtype 16[20]. Another high-risk subtype, HPV-18, is found in some anal tumors, but is less common than HPV-16[19,21].

Subtypes 6 and 11 are less likely to become malignant and are commonly found in anogenital warts[22]. Other subtypes, including HPV-31, 33, and 45, have been implicated in a small number of anal cancer cases and have been linked to vulvar, cervical, and vaginal cancers in females, and penile cancer in males[20,23,24]. Verruca vulgaris (common warts) are often associated with the low-risk subtypes (1, 2, 4, and 7), with occasional high-risk subtypes (16 and 18) as a cause [25]. Anal SCC is the most common histopathological subtype, and HPV is implicated in more than 80% of tumor samples [26].

Male circumcision is associated with lower rates of HPV infection and transmission[27,28]. A meta-analysis across 32 studies that analyzed the relationship between male circumcision and HPV infection rates found decreased odds of prevalence of HPV infections [odds ratio = 0.45; 95% confidence interval (CI): 0.34-0.61], reduced incidence rate of HPV infection (incidence rate ratio = 0.69; 95%CI: 0.57-0.83), and increased risk of clearing HPV infections (risk ratio = 1.44; 95%CI: 1.28-1.61) at the glans penis in male subjects[29]. The HPV vaccine and condoms are also implicated in protecting and reducing virus transmission rates[30,31]. However, condoms do not offer complete protection because they do not mask all areas of the body that may be susceptible to infection, such as the anus[30,31].

### *Human immunodeficiency virus infection*

Patients with human immunodeficiency virus (HIV) are considered high risk for anal cancer[32]. Incidence rates of anal carcinoma among HIV-infected individuals are 30 times higher compared to the general population[33]. A cohort study by Silverberg *et al*[34] that compared the incidence rates among 34189 HIV-infected [55% men who have sex with men (MSM), 19% other men, 26% women] and 114260 HIV-noninfected (90% men) individuals found the unadjusted incidence rate per 100000 person-years to be 131 for HIV-positive MSM, 46 for other HIV-infected men, and 2 for HIV-negative men. HIV-infected women had an anal carcinoma rate of 30 per 100000 person-years, with no cases observed for HIV-noninfected women[34]. The use of highly active retroviral therapy has not reduced the incidence of anal cancer, and a systematic review revealed a continued increase in the incidence of anal carcinoma among HIV-infected women, despite the use of highly active retroviral therapy[35,36].

### *Tobacco use*

Tobacco use is an independent risk factor for anal carcinoma[37]. It is associated with anal cancer disease recurrence and higher mortality rates[37,38]. A study conducted by Phillips *et al*[39], which measured smoking-related DNA adducts in the anal epithelium among smokers ($n$ = 20) and age-matched life-long non-smokers ($n$ = 16), showed high adduct levels among smokers compared to controls. The authors concluded that tobacco smoke inflicts genotoxic damage in the anal epithelium of smokers as a plausible mechanism for its association with anal cancer.

### *Chronic immunosuppression not due to HIV*

Compromised immune function is associated with increased risk for anal cancer[40]. According to a population-based cohort study conducted in Denmark by Sunesen *et al*[41], immunosuppression in the form of autoimmune diseases, solid organ transplantation, and hematologic malignancy are all strongly associated with the development of anal carcinoma.

### *Men who have sex with men*

MSM are 20 times more likely to be diagnosed with anal cancer than heterosexual men, with a rate of approximately 40 cases per 100000 people[42]. HIV-infected MSM are up to 40 times more likely to develop anal cancer, resulting in a rate of 80 cases per 100000 people[42,43]. The high incidence is related to significant HPV infection rates[43,44]. Modifiable behavioral factors such as condom and drug use can lower infection rates and cancer incidence[43].

### *Female sex*

Anal cancer has a predilection for females compared to males, most notably due to high HPV infection rates among the sex[45,46]. Several factors, including multiple sexual partners, sex at an early age, and having an uncircumcised sexual partner, all contribute to higher rates of HPV infection and, therefore, high rates of anal cancer compared to their male counterparts[45-47].

### *Older age*

Anal cancer is rare in people younger than 35 and is mainly found in older adults[48,49]. The development of anal carcinoma in older patients is primarily reflected by the accumulation of cell DNA damage over time[50].

## ANATOMY

The anal canal (Figure 1) is the terminal portion of the alimentary tract that extends from the anorectal junction to the anal margin[51]. The upper portion of the canal derives from the dorsal section of the cloaca (endoderm), and the lower part is derived from the proctodeum (ectoderm)[51,52]. The canal measures approximately 4 cm and is continual with the rectum at the anorectal junction that forms an angle (anorectal angle) at the levator ani[51-53]. The muscular layer of the canal shapes the internal and external anal sphincters, and the tube contains longitudinal folds of mucosa that join inferiorly to make semicircular anal valves[53,54]. These valves form the pectinate (dentate) line, which demarcates the superior two-thirds of the anal canal from the inferior one-third[55]. The pectinate line is a watershed area that varies in neurovascular supply[55,56]. The epithelium above this line is columnar, while below is a transition zone lined by

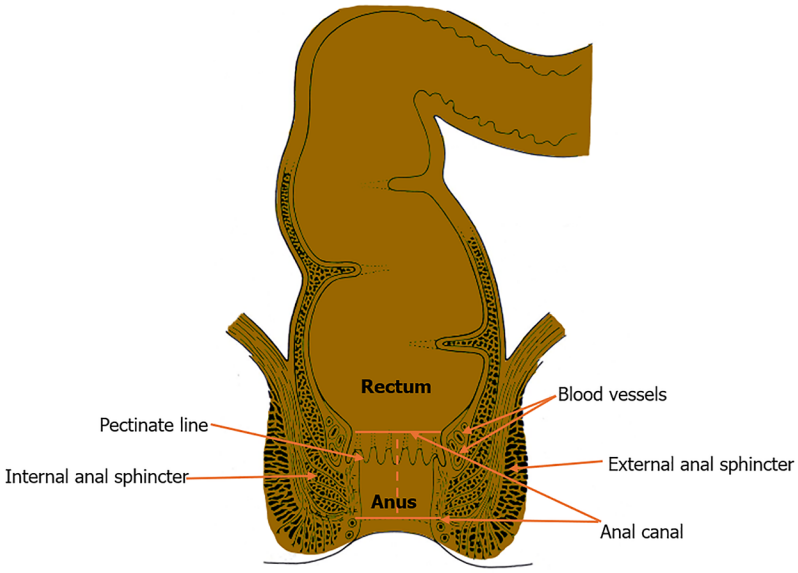


**Figure 1 Image depicting parts of the large bowel.** The anal canal is labeled as the terminal portion of the rectum.

nonkeratinized stratified squamous epithelium known as the anal pecten[57]. Further below, the anal pecten ends at the anocutaneous (white) line, where the epithelium resembles true skin (*i.e.* keratinized stratified squamous with sebaceous glands and hair)[57,58].

The anal canal above the pectinate line receives blood supply from the superior rectal artery (branch of the inferior mesenteric artery), median sacral arteries, and anastomosing branches from the middle rectal artery[55,56,59]. The venous drainage anastomoses with the rectal venous plexus and, above the line, drains from the superior rectal vein to the inferior mesenteric vein (portal venous system)[57-60]. Below the dentate line, the middle and inferior rectal veins drain to the internal pudendal vein, a tributary of the internal iliac vein[58-60]. Lymph drains primarily to the internal iliac nodes above the line, while inferiorly, it drains to the superficial inguinal nodes[60,61]. Above the pectinate line, the anal canal receives visceral innervation *via* the inferior hypogastric plexus[62]. As a result, the superior portion of the canal is susceptible to stretching[62,63]. Below the line, the canal receives somatic innervation through the inferior rectal branches of the pudendal nerves and, consequently, is sensitive to pain, temperature, and touch[63,64].

## PATHOPHYSIOLOGY

Integration of HPV double-stranded DNA into the host cell genome facilitates the expression of viral oncoproteins E6 and E7, promoting tumor oncogenesis of anal SCC[65]. These proteins express stimulatory properties, leading to a complex inflammatory progression to invasive cancer[65,66]. During integration, a destruction of a segment of the E2 domain of the viral genome occurs in the DNA of the infected cells, which leads to a loss of suppressor function of the E2 protein[65-67]. Due to this loss of activity, E6 and E7 proteins have increased expression with stimulatory properties, promoting invasion and keratinocyte immortalization[65-68].

HPV-related neoplasm requires E6 and E7 expressions to establish and maintain a transformed state[67,68]. Within this environment, the E7 complex interacts with the retinoblastoma protein, and E6 binds and inactivates tumor suppression protein p53[67-69]. This complex interplay serves as the primary mechanism for HPV-related anal SCC[65-69]. HIV-associated anal cancer is believed to be related to microsatellite instability, leading to the progression of invasive carcinoma[70].

## IMMUNIHISTOCHEMISTRY

Regarding immunohistochemistry (Table 1), SCC express cytokeratin (CK) 5/6, CK 13/19, pan CK antibody (AE1/AE3), and p63 protein[66-71]. The presence of the p63 protein on immunohistochemistry is highly specific for anal SCC[68-71]. Adenocarcinoma of the anal gland, in contrast, commonly expresses CK 7/20 (+/-)[67-71]. Adenoid cystic tumors are usually CK 7+, and melanocytic markers (*i.e.* S-100, human melanoma black-45, and Melan-A) are positive in melanomas [72,73]. Neuroendocrine tumors are positive for neuroendocrine markers (*i.e.* chromogranin A and serum neuron-specific enolase), and lymphoid cancers express lymphoid markers[74,75]. Perianal Paget disease commonly expresses CK 7 and gross cystic disease fluid protein 15[76]. Paget disease with associated anorectal cancer is typically CK 20+[76].

**Table 1 Immunohistochemistry markers for anal cancers**

| Tumor markers | Squamous cell carcinoma | Anal adenocarcinoma | Melanoma | Basal cell carcinoma | Neuroendocrine | Paget primary | Paget secondary |
|---|---|---|---|---|---|---|---|
| AE1/AE2 | + | + | - | + | + | + | + |
| Ber-EP4 | - | + | - | + | - | + | + |
| CAM5.2 | + | + | - | Nonspecific | + | + | + |
| CDX-2 | - | Mostly- | - | - | - | - | + |
| CEA | - | - | - | - | - | + | + |
| Chromogranin | - | - | - | - | + | - | - |
| CK5/6 | + | - | | + | - | - | - |
| CK7/20 | -/- | +/Mostly- | - | -/- | -/- | +/- | Mostly-/+ |
| GCDFP-15 | - | - | - | - | - | + | - |
| HMB45 | - | - | + | - | - | - | - |
| MELAN-A | - | - | + | - | - | - | - |
| Mucin | - | + | - | - | - | + | + |
| p63 | + | - | - | + | - | - | - |
| S100 | - | - | + | - | - | - | - |
| Synaptophysin | - | - | - | - | + | - | - |
| Vimentin | - | - | + | - | - | - | - |

AE1: Anti-pan cytokeratin AE1; Ber-EP4: Anti-epithelial cell adhesion antibody; CAM5.2: Anticytokeratin CAM5.2; CDX-2: Caudal-related homeobox transcription factor 2; CEA: Carcinoembryonic antigen; CK5/6: Cytokeratin 5/6; GCDFP-15: Gross cystic disease fluid protein 15; MELAN-A: Melanoma antigen recognized by T cells.

## PRECURSOR LESIONS

Anal cancer commonly originates at the squamocolumnar junction and arises from precancerous lesions termed anal intraepithelial neoplasia (AIN)[77]. These lesions are frequently called squamous intraepithelial lesions, and are grouped according to their grade and degree of dysplasia[77,78]. AIN 2/3 are commonly classified as high-grade squamous intraepithelial neoplasia and are found in most patients with anal cancer[77,78]. Consequently, patients who are at increased risk for AIN 2/3, which includes MSM, solid organ transplant recipients, immunocompromised patients, and women with any history of vulvar, vaginal, and cervical cancer should undergo regular screening with anal cytology[77-79].

AIN has been found to be biologically similar to cervical intraepithelial neoplasia (CIN) due to its common association with HPV, which is found in most cancers of the anus and cervix[80]. Current modes of treatment include topical medications, laser therapy, fulguration, and ablative techniques in the form of infrared coagulation, surgical excision, and thermal ablation. Topical treatment options include imiquimod, 5-fluorouracil (5-FU), and trichloroacetic acid[81].

## PATHOLOGY

The World Health Organization has classified tumors of the anal canal into intraepithelial and invasive neoplasms that are further divided into epithelial and nonepithelial tumors (Table 2). The most notable ones are as follows.

### *Paget disease*

Anal Paget disease commonly involves the perianal skin and is found in apocrine-rich areas[76,82]. Malignant cells produce mucus, and perianal spread is characterized by the proliferation of pagetoid cells within the epithelium[76,82,83]. Tumor cells on histological appearance often show large cells with giant nuclei and pale cytoplasm[82-84]. Pagetoid cells also occasionally acquire a signet ring appearance[82-85].

### *SCC*

More than 80% of anal cancers are SCC[3,4]. These tumors arise primarily at the transformation zone between the columnar and squamous epithelium of the canal[3,4,33-36]. The cells that constitute these tumors have varying features, with some having giant pale eosinophilic characteristics and others having no areas of keratinization[86]. Other cells facilitate tumor nests (tumor-cell islands) that may appear with central keratinization and intercellular bridges or

**Table 2 World Health Organization histological classification of anal tumors**

| Tumor type | Characteristics |
|---|---|
| Epithelial tumors | |
| Premalignant lesions | Intraepithelial neoplasia (dysplasia), low-grade |
| | Intraepithelial neoplasia (dysplasia), high-grade |
| | Bowden disease |
| | Perianal squamous intraepithelial neoplasia |
| | Paget disease |
| Carcinoma | Squamous cell carcinoma |
| | Adenocarcinoma |
| | Mucinous adenocarcinoma |
| | Small cell carcinoma |
| | Undifferentiated carcinoma |
| | Others |
| Nonepithelial tumors | Leiomyoma |
| | Gastrointestinal stromal tumor |
| | Myofibroma |
| Carcinoid tumors | NA |
| Melanoma | NA |
| Secondary tumors | Direct extension from adjacent organs: Rectal, cervical, and prostate carcinoma |

NA: Not applicable.

peripheral palisading on histology[86,87]. SCC tumors lack a myoepithelial layer within and around the basement membrane, and may resemble adnexal, skin, and salivary gland neoplasms[86,87].

### *Adenocarcinoma*

Adenocarcinoma accounts for approximately 5% to 10% of cancers of the anal canal[4,88,89]. This histological tumor type primarily originates from the anal epithelium and includes primary adenocarcinomas that arise in the mucosa, anal glands, and fistula[89,90]. These tumors may also appear as small, ulcerated lesions near the anal duct, and possess some association with ulcerative colitis, Crohn's, and Paget's disease[89-92]. Tumors are commonly mucin-positive and are treated similarly to rectal cancer[4,89-92].

### *BCC*

BCC, the rarest type of anal cancer, accounts for less than 0.2% of anal neoplasms and predominantly affects the skin around the anal region[93,94]. It arises from perianal lesions and tends to remain local[93,94]. Anal BCC shares similar histological features with a known variant of SCC[94]. This variant, termed SCC with basaloid features, tends to show nests of oval cells with varying ratios of eosinophilic to basophilic cytoplasm, assorted mitotic activity, and peripheral nuclear palisading[94-96]. Perianal BCC has a better prognosis than SCC with basaloid features, and the nodular subtype is the predominant histologic type found in most cases[94-97].

### *Carcinoid tumors*

Carcinoid tumors of the anal canal (rectal carcinoid) are rare and account for 1% to 1.3% of all anal cancers[98]. These tumors are commonly asymptomatic and are found incidentally on routine colonoscopy[98,99]. Rectal carcinoids are of neuroendocrine origin and are well differentiated[99]. Due to this fact, they are associated with a favorable prognosis, although they can metastasize[98,99]. This ability to spread is more likely with a tumor size greater than 10 mm and atypical features[99,100]. On histological appearance, these tumors show a trabecular growth pattern with nest- or rose-like structures[100,101]. Immunohistochemistry markers for neuroendocrine tumors include chromogranin A, synaptophysin, and neuro-specific enolase. However, the most sensitive marker for rectal carcinoids is SABT2, which is positive in more than 85% of rectal carcinoids[101,102].

### *Melanoma*

Anal melanomas are rare and account for only 1% of all anorectal malignant tumors[103]. These are often acro-lentiginous

and are usually found as pigmented ulcerated lesions[103,104]. Melanomas of the anal canal are typically aggressive and exhibit poor prognosis due to the lack of symptoms and late disease presentation[105]. The histological types of anal melanoma include lymphoma-like, spindle-cell, epithelioid, and pleomorphic[103-105]. The spindle-cell anal melanoma can be easily misdiagnosed as a gastrointestinal stromal tumor[105,106]. For this reason, analysis of immunohistochemistry is vital for the correct diagnosis[105]. Anal melanoma most commonly expresses S-100 on immunohistochemistry and c-Kit positive in over 70% of cases[103-106].

## DIAGNOSTIC WORK-UP

Most patients with anal cancer typically present with symptoms resembling benign diseases such as fissures and hemorrhoids[4,107]. Patients may complain of abdominal/rectal pain and bleeding on toilet paper after wiping[4,107]. In other cases, patients may be asymptomatic[4,107,108]. A complete history and physical examination are crucial to prognosis as patients with early diagnosis have better clinical outcomes[108-110]. Physicians should have a high index of clinical suspicion for patients presenting with symptoms resembling benign disease, and possess a low threshold for imaging studies and biopsy of suspected lesions[107-111]. Imaging modalities used in the diagnosis of anal cancer are as follows.

### *Three-dimensional endoanal ultrasound*

Three-dimensional endoanal ultrasound (3D-EAUS) is a valuable tool that allows a detailed evaluation of the anatomy and diseases of the anal canal[112]. It can accurately assess the depth of invasion of cancer into the anal sphincter complex and gauge tumor response to CRT[112,113]. 3D-EAUS is easy to perform and reproduce with high diagnostic accuracy [112-114]. A large study by Reginelli *et al*[114], which compared the diagnostic performance of 3D-EAUS and magnetic resonance imaging (MRI) in the detection of anal cancer, found a detection rate of 100% using ultrasound compared to 93.1% for MRI.

### *Computed tomography*

Computed tomography (CT) scan is a valuable tool in the diagnosis of anal cancer[115]. Contrast-enhanced CT scan may visualize an anal tumor as a hypo-attenuated necrotic mass[5,115]. CT scan is also helpful in evaluating lymph node metastases and distant metastatic disease[115,116]. Although useful in the diagnostic work-up of anal cancer, recent guidelines have geared toward better imaging modalities such as MRI, positron emission tomography (PET), and PET/CT scan[115-117]. A systematic review and meta-analysis by Mahmud *et al*[117] comparing PET/CT or PET with standard imaging studies showed a 67% sensitivity to detect primary tumors with CT scan alone compared to 99% for PET or PET/CT scan.

### *Phased-array MRI*

Phased-array MRI is a useful tool for the diagnosis and staging of anal cancer[118]. In 2014, the European Society of Medical Oncology guidelines proposed that pelvic MRI should be mandatory in the diagnostic work-up of anal cancer, citing superior soft-tissue resolution compared to conventional (standard) imaging modalities (*i.e.* 3D-EAUS and CT scan) [119]. MRI detects neoplastic nodes, the extent of tumor infiltration into nearby organs, and the circumferential tumor extent[119,120]. It is greater than 90% sensitive in identifying anal carcinoma, and provides specific detail regarding the position of the tumor[120].

### *PET/CT scan*

PET/CT scan with 18F-fluorodeoxyglucose (FDG-PET/CT) is an accurate and effective imaging modality for the detection and staging of anal cancer[121]. PET detects tumors based on molecular alterations and plays a vital role in post-treatment restaging of anal cancer patients[121,122]. FDG-PET/CT provides details regarding crucial markers for staging, such as the size of the tumor, the lymph nodes involved, and the detection of metastasis[121-123]. Of note, FDG-PET/CT is particularly sensitive for localized tumors less than 2 cm compared to standard imaging modalities[5,121-123].

## STAGING

The tumor, node, and metastasis (TNM) classification system (Table 3) published by the American Joint Committee on Cancer is used to stage anal cancer[124]. This system is based on three essential factors.

### *Tumor*

Tumor (T) describes the extent of the primary tumor and its size in relation to growth into nearby structures or organs [124,125]. A larger value after the T reflects a bigger tumor size and growth into nearby structures[125]. TX indicates that the primary tumor cannot be assessed due to lack of information, and T0 means there is no evidence of a primary tumor [120,125]. T1 indicates tumor sizes less than 2 cm in the greatest dimension, T2 reflects tumor size between 2 and 5 cm, and T3 indicates tumor sizes more than 5 cm[124]. Lastly, T4 represents a tumor of any size that invades adjacent organs [120,124,125].

**Table 3 Tumor, node, and metastasis classification system for anal cancer**

| Primary tumor | Characteristics |
|---|---|
| TX | Primary tumor cannot be assessed |
| T0 | No evidence of primary tumor |
| Tis | HSIL (previously termed carcinoma *in situ*, Bowden disease, anal intraepithelial neoplasia II-III, high-grade anal intraepithelial neoplasia) |
| T1 | Tumor ≤ 2 cm in greatest dimension |
| T2 | Tumor > 2 cm but ≤ 5 cm in greatest dimension |
| T3 | Tumor > 5 cm in greatest dimension |
| T4 | Tumor of any size invades adjacent organ(s) (*e.g.*, vagina, urethra, bladder) |
| Lymph nodes | |
| NX | Regional lymph nodes cannot be assessed |
| N0 | No regional lymph node metastasis |
| N1 | Metastasis in inguinal, mesorectal, internal iliac, or external iliac nodes |
| | N1a: Metastasis in inguinal, mesorectal, or internal iliac lymph nodes |
| | N1b: Metastasis in external iliac lymph nodes |
| | N1c: Metastasis in external iliac with any N1a nodes |
| Metastasis | |
| M0 | No distant metastasis |
| M1 | Distant metastasis |

HSIL: High-grade squamous intraepithelial lesion.

### *Regional lymph node*

Lymph node (N) describes the spread of cancer to nearby lymph nodes[120,124]. NX indicates that regional lymph nodes cannot be assessed due to insufficient information[120,124]. N0 means no regional lymph node metastasis, and N1 indicates metastasis to nearby lymph nodes, such as the internal or external iliac nodes[120,124,125].

### *Distant metastasis*

Metastasis (M) describes the spread of cancer to distant lymph nodes or organs[124]. M1 represents distant metastasis [120,124]. Anal cancer is staged based on TNM status (Table 4)[124]. Stage I and II are localized diseases[120,124]. Stage III indicates locally advanced cancer and stage IV represents metastatic disease[120,124,125].

## TREATMENT OF LOCOREGIONAL ANAL CANCER

Anal SCC was predominantly treated with radical resection before the 1970s[121-125]. This procedure involved the removal of the distal colon, rectum, anus, and anal sphincter complex through perineal and anterior abdominal wall techniques, which formed a permanent colostomy[126]. This procedure resulted in 5-year overall survival (OS) rates between 40% and 70% and a surgical mortality rate of 3%[127].

A shift in the approach to the treatment of anal SCC came when researchers at Wayne State University in 1974 investigated a therapeutic regimen for SCC consisting of chemotherapy and radiation[128]. This protocol (the Nigro regimen) consisted of 5-FU, fluoropyrimidine, and mitomycin-C (MMC) with a concurrent dose of gray[128]. The results were promising, as the first three patients treated with the regimen experienced complete tumor regression[128-130]. A subsequent follow-up study was conducted in which patients treated with the protocol only underwent a salvage APR if there was clinical evidence of disease residue[130]. The 5-year OS rate was 67%, and the 5-year colostomy-free survival rate was 59%. A total of 84% of patients in the follow-up study showed complete response to CRT.

These results sparked further investigation into the role of CRT in the management of anal cancer, and several randomized controlled trials (RCTs) have since been done to cement CRT as the definitive treatment for anal cancer[131-135]. These trials have investigated the benefits of CRT *vs* radiotherapy (RT) alone, sequencing, and optimal chemotherapy protocols with simultaneous RT, and high-dose RT.

**Table 4 Anatomic stage of anal cancer**

| Stage | T | N | M |
|---|---|---|---|
| 0 | Tis | N0 | M0 |
| I | T1 | N0 | M0 |
| IIA | T2 | N0 | M0 |
| IIB | T3 | N0 | M0 |
| IIIA | T1-T2 | N1 | M0 |
| IIIB | T4 | N0 | M0 |
| IIIC | T3-T4 | N1 | M0 |
| IV | Any T | Any N | M1 |

M: Metastasis; N: Node; T: Tumor.

### *CRT vs RT*

The United Kingdom Coordination Committee on Cancer Research phase 3 RCT, which involved 585 patients with anal SCC, compared RT to CRT[136]. The trial concluded that the use of 5-FU and MMC in combination with RT resulted in a 46% risk reduction in the local failure rate [relative risk (RR) = 0.54, 95%CI: 0.42-0.69], with a local failure rate of 36% with CRT *vs* 59% with RT. CRT was also associated with a significant reduction in cancer mortality, citing a 3-year anal cancer mortality of 39% with RT compared to 28% with CRT (RR = 0.71, 95%CI: 0.53-0.95). A RCT by the European Organization for Research and Treatment compared the use of RT or CRT (5-FU and MMC) in patients with anal cancer[135]. CRT was found to be associated with more improvement in local and regional control rates. A 32% difference in colostomy-free time was observed between the two groups, and an 18% difference in local and regional failure rates.

### *Chemotherapy protocol and sequence*

MMC, although associated with toxicity, is an essential drug in chemotherapy regimens[137]. A phase 3 intergroup RCT that compared combined 5-FU and MMC to 5-FU alone showed a favorable colostomy-free rate (71% *vs* 59%, $P = 0.014$) and disease-free survival (DFS) (73% *vs* 51%, $P = 0.0003$) with combination chemotherapy at 4 years[134]. The addition of MMC to 5-FU, however, was associated with a greater risk of high-grade toxicity (23% *vs* 7%, $P \leq 0.001$). Two RCTs then compared MMC to combined cisplatin and 5-FU, which revealed conflicting results[132,133]. The United States gastrointestinal (GI) intergroup radiation therapy oncology group (RTOG) 98-11 phase 3 trial randomized anal cancer patients to CRT including 5-FU and MMC *vs* two induction cycles of 5-FU and cisplatin followed by CRT including 5-FU and cisplatin[133]. The 5-FU and MMC combinations were associated with better 5-year DFS and OS than the 5-FU and cisplatin combinations. DFS was 68% for 5-FU and MMC compared to 58% for 5-FU and cisplatin at 5 years; OS was 78% and 71%, respectively. The overall severity of toxicity was similar between the treatment arms. The results of RTOG 98-11 continued to support the use of combined MMC and 5-FU with RT for patients with anal SCC.

A 2 × 2 factorial trial, ACT II, conducted by the United Kingdom Coordination Committee on Cancer Research, also investigated the substitution of MMC with cisplatin[132]. Patients with anal cancer were first randomized to receive CRT with either MMC or cisplatin, followed by randomization to receive adjuvant therapy with 5-FU and cisplatin or observation. Both arms had similar complete response rates at approximately 90%. Toxicity rates were about 70%, with comparable OS and DFS. The progression-free survival was 74% for maintenance chemotherapy *vs* 73% for no maintenance at 3 years. The results of the ACT II and RTOG 98-11 trials have together solidified 5-FU and MMC as the standard of care for managing locoregional anal SCC[132,133]. Several studies have been published recently, which highlight that capecitabine and MMC appear to have equal efficacy to infusional 5-FU and MMC in the treatment of locally advanced anal SCC[138-140].

### *Intensity modulated RT*

Intensity modulated RT (IMRT) is the preferred method for RT in the treatment of locoregional anal SCC[141]. This modality utilized variations in radiation intensities that facilitate more precise mapping of tumor targets and spares nearby structures[141,142]. The goals of treatment are to optimize radiation dose and duration to limit toxicity and locoregional recurrence[141,143]. IMRT is preferred over conventional RT based on the results of a phase two multicenter prospective RCT (RTOG 0529) that compared 3D-conformal RT to dose-painted IMRT[144]. The primary endpoint was a decrease in the combined rate of grade 2 + GI and genitourinary adverse events by at least 15% compared to previous results in the RTOG 98-11 trial[133,144]. Significant reductions in grade 2 hematologic, grade 3 dermatologic, and GI toxicity were observed in the IMRT group. However, despite the improved toxicity profile, retrospective studies have failed to show a difference in survival outcomes between cancer patients who received standard RT *vs* IMRT[145,146].

### *Surgery*

In locoregional anal cancer, CRT should be exhausted as the number one option for treatment due to better outcomes

historically *vs* surgery[147,148]. APR and the formation of end colostomy should be reserved for salvage therapy in patients with tumor progression, disease recurrence following CRT, or patients who are ineligible for definitive CRT[148, 149]. The exception to CRT as a first-line treatment for locoregional cancer is early-stage (stage I disease) perianal disease excluding the anal sphincter and superficially invasive anal SCC, for which a wide local excision is a treatment option [126,141].

## TREATMENT OF RECURRENT AND METASTATIC DISEASE

### *Locoregional disease recurrence*

Recurrent disease is defined as tumor discovery after 6 months post-CRT and a residual tumor if present within 6 months of treatment[150,151]. The average time for tumor recurrence of anal cancer is less than 12 months after CRT, and suspicion should prompt a detailed work-up with imaging and biopsy of all lesions to exclude metastasis[150]. The goal of management is to attain negative tumor margins, and patients are treated with APR with a permanent end colostomy and the creation of a sizable pelvic floor defect[151,152]. Salvage APR results in the control of disease locally in approximately 60% of all cases[151-153]. Salvage surgery also has a 5-year OS rate between 30 and 60%[154,155]. RT or brachytherapy following CRT has limited use in the management of local disease recurrence[141,156]. Similarly, the use of immunotherapy or systemic chemotherapy has limited data, and further research needs to be done to support use, although some articles have justified the use of MMC in the definitive management of local disease recurrence despite higher toxicity levels[141,157,158].

### *Metastatic disease recurrence*

Systemic therapy is the current standard of treatment for patients with metastatic cancer or metastatic disease recurrence [159]. More than 10% of patients commonly experience distant relapse following CRT, and less than 10% of patients with *de novo* metastatic disease[159,160]. This first-line therapy is a platinum-based doublet regimen with either cisplatin or carboplatin and 5-FU or paclitaxel[161,162]. The phase 2 InterAACT trial randomized cancer patients with advanced anal SCC to treatment with either carboplatin and weekly paclitaxel or infusional 5-FU and cisplatin. Results suggested improvements in OS (20 months *vs* 12 months, $P = 0.01$) with the carboplatin/paclitaxel regimen and a favorable toxicity profile (36% *vs* 62% adverse event rate, $P = 0.02$) in comparison to the cisplatin and 5-FU regimen[163]. This trial established carboplatin/paclitaxel as the primary therapy for patients with metastatic disease and disease recurrence, and remains the standard of treatment for patients in the United States. Other studies have been done on regimens such as docetaxel, cisplatin, and 5-FU (DCF) and 5-FU, leucovorin, and cisplatin (FOLFCIS)[164,165]. These studies have shown promising results, making DCF and FOLFCIS good alternative options for patients with metastatic disease.

## SURVEILLANCE FOLLOWING PRIMARY TREATMENT

According to guidelines from the National Comprehensive Cancer Network, patients should be re-evaluated with a digital rectal examination and anoscopy 8 weeks to 12 weeks after completion of CRT[166]. Following evaluation, patients should be classified according to whether they have complete remission of the disease, persistent disease, or progressive disease.

Patients with complete remission should have digital rectal examinations and lymph node exams every 3 months to 6 months for 5 years after treatment. Anoscopy is also recommended every 6 months to 12 months for 3 years post-treatment. Imaging is suggested for stage II and III anal cancers in complete remission every year for 3 years and takes the form of a CT of the chest, abdomen, and pelvis with contrast or a CT of the chest without contrast, plus an MRI of the abdomen and pelvis with contrast.

Patients who are found to have remaining cancer that has not grown, or spread are classified as having persistent disease. Watchful waiting for a maximum of 6 months can be considered, as some anal cancers take longer to respond to treatment. Following this period, the patient should be reassessed, and then be categorized as progressive disease if there is persistent cancer or worsening following this period. If the cancer goes into remission, surveillance follows the complete remission protocol.

Lastly, if a follow-up examination 8 weeks to 12 weeks after CRT finds that the cancer has grown or spread, the patient should be classified as having progressive disease, and a biopsy should be performed. If biopsy results confirm progression, imaging (CT scan with contrast or non-contrast CT and MRI) should be done to assess the location and spread of the disease, and to determine treatment.

## PREVENTION AND SCREENING

Anal cancer is a rare disease that accounts for approximately 0.5% of all new cancers in the United States and less than 3% of cancers of the intestine[4,167,168]. While the incidence of anal cancer is too low for general population screening, there is a specific subset of groups with increased risk for AIN 2/3, including HIV-positive patients, MSM, solid organ transplant recipients, women with any history of cervical, vulvar, or vaginal cancer, and immunocompromised patients not due to HIV[167-169]. The high burden of anal cancer in these populations is partly attributed to the higher prevalence

of HPV infection[169,170]. As a result, these patients should be regularly screened with anal cytology (also termed anal pap testing, using a Dacron swab inserted into the anal canal)[171,172]. Patients with an abnormal pap test should then undergo frequent surveillance with high-resolution anoscopy[173,174].

A single-center prospective study that evaluated the diagnostic accuracy of early anal cancer detection in high-risk patients (AIN-2+) using combined anal pap testing and high-resolution anoscopy showed that the diagnostic accuracy of anal cancer improves when the two tests are combined in comparison to anal pap testing alone[175].

HPV vaccination is a proven and cost-effective approach to the prevention of anal cancer in the younger population [176-178]. The administering of the 9-valent HPV vaccine to children could effectively prevent almost all anal tumors (primary prevention of anal SCC)[177]. Furthermore, the vaccine may help prevent AIN 2/3 and possible progression to SCC in high-risk groups[176-178]. This is true for populations that include MSM, HIV-positive men and women, solid organ transplant recipients, and women with a history of cervical cancer or CIN 3[177,179].

## FUTURE DIRECTIONS

Although significant progress has been made with respect to the diagnosis and management of anal cancer, knowledge gaps still exist. More research is needed to address the prevention of anal carcinoma, especially in patients who are deemed high risk. Additionally, research focusing on minimizing the adverse effects of CRT in patients with early disease is crucial to improving the quality of life and survivorship care. Immunotherapy is an active area of research in the treatment of anal SCC. Therapeutic agents targeting programmed cell death receptor 1 have been shown to be effective against other HPV-related cancers, such as cutaneous SCC of the skin[6,180]. Due to this fact, several clinical trials are currently ongoing to investigate the efficacy of anti-programmed cell death receptor 1 agents in both metastatic and localized anal cancer (Table 5). Investigators are also currently assessing the role of monoclonal antibodies for use in anal cancer after the success of anti-epidermal growth factor receptor (anti-EGFR) therapies for cancers of the head and neck [181-183]. Cetuximab, an EGFR inhibitor combined with an interleukin-15 receptor superagonist, is currently being studied in the treatment of advanced SCC[184].

**Table 5 Ongoing clinical trials investigating the use of anti-programmed cell death-1 agents in anal squamous cell carcinoma**

| NCT number | Official title | Interventions | Study type | Phase | Status | Location |
|---|---|---|---|---|---|---|
| NCT04719988 | Ezabenlimab (BI 754091) and mDCF (Docetaxel, Cisplatin, and 5-fluorouracil) Followed by Chemoradiotherapy in Patients With Stage III Squamous Cell Anal Carcinoma. A Phase II Study | Biological: Blood sample collection<br>Procedure: Biopsy | Interventional | Phase 2 | Active | France |
| NCT04894370 | Spartalizumab, mDCF (Docetaxel, Cisplatin, and 5-fluorouracil) and Radiotherapy in Patients With Metastatic Squamous Cell Anal Carcinoma. A Phase IIA Study | Biological: Sample collection | Interventional | Phase 2 | Recruiting | France |
| NCT04708470 | A Phase I/II Study of Combination Immunotherapy for Advanced Cancers Including HPV-Associated Malignancies, Small Bowel, and Colon Cancers | Drug: Bintrafusp Alfa<br>Drug: NHS-IL12<br>Drug: Entinostat | Interventional | Phases 1 and 2 | Recruiting | United States |
| NCT04432597 | Phase I/II Trial of HPV Vaccine PRGN-2009 Alone or in Combination With Anti-PD-L1/TGF-Beta Trap (M7824) in Subjects With HPV-Positive Cancers | Biological: PRGN-2009 (Phase I)<br>Biological: PRGN-2009 (Phase II)<br>Biological: M7824<br>Diagnostic test: MRI<br>Diagnostic test: Bone scan<br>Diagnostic test: CT scan<br>Diagnostic test: Brain CT<br>Diagnostic test: Brain MRI<br>Procedure: Biopsy (Phase I) | Interventional | Phases 1 and 2 | Active | United States |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | Procedure: Biopsy (Phase II) | | | | |
| NCT05544929 | A Phase I, Open-label, Multicenter Study of KFA115 as a Single Agent and in Combination With Pembrolizumab in Patients With Select Advanced Cancers | Drug: KFA115<br>Drug: Pembrol-izumab | Interventional | Phase 1 | Recruiting | United States |
| NCT04357873 | Phase II Basket Trial Evaluating the Efficacy of a Combination of Pembrolizumab and Vorinostat in Patients With Recurrent and/or Metastatic Squamous Cell Carcinoma | Drug: Pembrol-izumab; vorinostat | Interventional | Phase 2 | Active | France |
| NCT04802876 | Efficacy of Tislelizumab and Spartalizumab Across Multiple Cancer-types in Patients With PD1-high mRNA Expressing Tumors Defined by a Single and Pre-specified Cutoff | Drug: Spartalizumab<br>Drug: Tislelizumab | Interventional | Phase 2 | Recruiting | Spain |

CT: Computed tomography; HPV: Human papillomavirus; mDCF: Modified DCF regimen; MRI: Magnetic resonance imaging; PD1: Programmed cell death 1; PD-L1: Programmed cell death ligand 1; TGF: Transforming growth factor.

Additional novel therapies, including triplet immunotherapy and HPV-targeted vaccines, are presently being studied in clinical trials. Pembrolizumab, in combination with a bifunctional EGFR/transforming growth factor β fusion protein, is actively being investigated in the phase I KEYNOTE-E28 clinical trial in patients with recurrent or metastatic SCC[185]. UCPVax, derived from telomerase, is a CD4 helper T-inducer vaccine currently being studied in combination with immune checkpoint inhibitors for HPV-positive malignancies, including SCC, in the VolATIL trial[186]. Lastly, circulating tumor DNA is an emerging tool being investigated in the current NOAC9 phase 2 clinical trial[187]. This trial compares standard surveillance *vs* HPV-positive circulating tumor DNA-guided image follow-up to assess for improved detection of early treatment failure or recurrence.

## CONCLUSION

Although anal cancer is rare in the general population, its incidence has been steadily increasing over the past decade. SCC is the predominant subtype of anal cancer and is HPV-mediated in most cases. The overall management of anal cancer has evolved over the past few decades. Currently, CRT, with the use of 5-FU and MMC, is the standard treatment for patients with early and locoregional disease. Wide local excision is a treatment option for a small subset of patients with stage I disease. Recurrence occurs in more than 20% of patients and requires salvage APR. A platinum-based doublet regimen is the mainstay of treatment for patients with metastatic disease. Immunotherapy is a treatment option for disease progression, and HPV vaccination is an effective long-term approach to limiting HPV-related anal cancer, especially in younger patients. Physical examination, in conjunction with routine screening, may increase detection of anal cancer in high-risk patients, which ultimately may reduce patient morbidity and mortality.

## FOOTNOTES

**Author contributions:** English KJ wrote the original draft, contributed to conceptualization, writing, reviewing, and editing of the manuscript; The author read and approved the final version of the manuscript.

**Conflict-of-interest statement:** The author reports no relevant conflicts of interest for this article.



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**S-Editor:** Wang JJ
**L-Editor:** Filipodia
**P-Editor:** Yu HG

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*World J Exp Med* 2024 September 20; 14(3): 95512

DOI: 10.5493/wjem.v14.i3.95512

ISSN 2220-315x (online)

*MINIREVIEWS*

# Phytochemical analysis of *Tinospora cordifolia* and *Withania somnifera* and their therapeutic activities with special reference to COVID-19

Prateek Rai, Tanya Garain, Deepshikha Gupta

**Specialty type:** Tropical medicine

**Provenance and peer review:** Invited article; Externally peer reviewed.

**Peer-review model:** Single blind

**Peer-review report's classification**

**Scientific Quality:** Grade C

**Novelty:** Grade B

**Creativity or Innovation:** Grade B

**Scientific Significance:** Grade C

**P-Reviewer:** Nakaji K

**Received:** April 11, 2024

**Revised:** July 19, 2024

**Accepted:** July 29, 2024

**Published online:** September 20, 2024

**Processing time:** 139 Days and 14.3 Hours

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## Abstract

Various important medicines make use of secondary metabolites that are produced by plants. Medicinal plants, such as *Withania somnifera* and *Tinospora cordifolia*, are rich sources of chemically active compounds and are reported to have numerous therapeutic applications. The therapeutic use of medicinal plants is widely mentioned in Ayurveda and has folkloric importance in different parts of the world. The aim of this review is to summarize the phytochemical profiles, folkloric importance, and primary pharmacological activity of *W. somnifera* and *T. cordifolia* with emphasis on their action against the novel coronavirus.

**Key Words:** Phytochemical analysis; *Tinospora cordifolia*; *Withania somnifera*; COVID-19; Immunomodulators; Giloy; Ashwagandha



**Core Tip:** This review provides insights into the phytochemical profiles and therapeutic activities of *Tinospora cordifolia* and *Withania somnifera*, which are medicinally significant plants of great importance in the practice of traditional medicine. Currently, the potential of these plants as antiviral agents against severe acute respiratory syndrome coronavirus (SARS-CoV), particularly SARS-CoV-2, is of interest.

**Citation:** Rai P, Garain T, Gupta D. Phytochemical analysis of *Tinospora cordifolia* and *Withania somnifera* and their therapeutic activities with special reference to COVID-19. *World J Exp Med* 2024; 14(3): 95512
**URL:** https://www.wjgnet.com/2220-315x/full/v14/i3/95512.htm
**DOI:** https://dx.doi.org/10.5493/wjem.v14.i3.95512

## INTRODUCTION

Immunomodulators are natural or synthetic chemical substances that are used to treat infectious diseases by modifying and regulating the immune system, which is the first barrier. Immunomodulators are generally grouped into three categories by their activity as immunosuppressants, immunostimulants, and immunoadjuvants[1]. Cytotoxicity and side effects reported to be associated with immunomodulators have led to the increased use of plant products for medicinal purposes in recent years. Plants have long been used for medicinal and therapeutic purposes. Pharmacologically active secondary metabolites produced by medicinal plants include alkaloids, steroids, glycosides, flavonoids, polyphenols and terpenoids[2-6]. In Ayurvedic literature, these medicinal plants are regarded as "Rasayana" and are known as "Amrita," which indicates they have significant medical properties and uses[7-12]. These plants have been analyzed for the presence of chemical compounds, such as those mentioned above, that can be used in the synthesis of drugs with target activity. Phytochemicals are isolated from dried plant material with various solvents including alcohols, ether, dichloromethane, or chloroform[13-16]. Plant-based nutraceuticals can aid in the prevention of viral invasion. Glutathione and functional amino acids (such as arginine, cysteine, glutamate, glutamine, glycine, taurine, and tryptophan), which are plentiful in food derived from animals, are essential for both human and animal health and immunity.

*Tinospora cordifolia* is a climbing plant in family *Menispermaceae* and grows throughout the Indian subcontinent and in some African countries *T. cordifolia* is important because of its medicinal properties. In Hindi, it is called giloy, which in Hindu mythology is a potion that helped the Gods stay permanently young[17-23]. *Withania somnifera*, known as winter cherry, or Indian ginseng[24], is a xerophytic plant in family *Solanaceae* and is found in arid areas and at altitudes above 5500 feet in the Himalayas. Strong immunomodulation by extracts of *W. somnifera* have been reported and were associated with activation of macrophages that are involved in the destruction of various pathogens like bacteria, fungi, viruses, *etc*[6,25-29].

A comprehensive search of PubMed Central, Scopus, and Google Scholar of all important articles published on *T. cordifolia* and *W. somnifera* from 1996 until the writing of this review manuscript was performed. Duplicate articles were merged. The primary outcome of this review was re-establishing the medicinal importance of these two plants, with reference to the important constituents present in their extracts. Their medicinal properties were attributed to the alkaloids, terpenoids, phenolics, flavonoids, and saponins that they contain. The potential of these plants to be used as antiviral agents against severe acute respiratory syndrome coronavirus (SARS-CoV), and SARS-CoV-2 in particular, was the secondary outcome.

## DETECTION OF VARIOUS CLASSES OF PHYTOCHEMICALS FROM *T. CORDIFOLIA* AND *W. SOMNIFERA*

*T. cordifolia* and *W. somnifera* contain various classes of phytochemicals that are responsible for their role in Aryuvedic and Unani medicine[6,30]. Table 1 describes the various classes of phytoconstituents reported to be present in both *T. cordifolia* and *W. somnifera* extracts. Both medicinal plants were found to contain alkaloids, phenolics, saponins, glycosides, steroids, phytosterols, flavonoids, carbohydrates, and amino acids as the major classes of natural products. These classes were evaluated by diagnostic testing as mentioned in Table 1[31-54]. Figures 1 and 2 show some of the important phytoconstituents responsible for the broad pharmacological activity of *T. cordifolia* and *W. somnifera*. Xia *et al*[43] recently attempted to list all the withanolides present in all the *Withania* species.

## PHARMACOLOGICAL ACTIVITY OF *T. CORDIFOLIA* AND *W. SOMNIFERA*

The literature available on the therapeutic uses of *T. cordifolia* indicates that it possesses important medicinal properties, including immunostimulatory, microbial, antioxidant, and hepatoprotective activities. It has been reported that glycosides such as cordifolioside A and B and syringin are the compounds primarily responsible for the immunomodulatory activity of *T. cordifolia*[18,22,35,40,55-60]. Tinocordiside[50], cordioside, and palmatosides were found to have neuroprotective activity in Parkinson's disease and dementia[44-50], and borapetoside C had antidiabetic activity[61]. The immunomodulatory substances included 11-hydroxymustakone, N-methyl-2-pyrrolidone, N-formylannonain, cordiofolioside A, and syringin. Furanolactone, and tinosporides were reported to have anti-inflammatory action in viral diseases, and tinosporin, isocolumbin, palmatine, and berberine had anticancer activity[49].

It has been reported that withanolides with steroidal cores account for the primary pharmacological activity of *W. somnifera* and are believed to be precursors in the synthesis of hormones. Withaferin A and withanolide D are examples of such compounds[51-54,62-65]. Withanolides were found to be potent anticancer, anti-inflammatory, antibacterial, and

**Table 1 Various classes of phytoconstituents reported to be present in both *Tinospora cordifolia* and *Withania somnifera* extracts and their diagnostic tests**

| S/No. | Compound class | Tests | *Tinospora cordifolia* | | | | | *Withania somnifera* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Extract types | A | B | C | D | E | A | B | C | D | E |
| 1 | Alkaloids | Mayer's test | √ | √ | √ | √ | X | X | X | X | √ | X |
| | | Dragendorff's test | √ | √ | √ | √ | X | X | X | X | √ | X |
| | | Hager's test | √ | √ | √ | √ | X | X | X | X | √ | X |
| 2 | Glycosides | Legal's test | X | X | √ | √ | X | X | X | √ | √ | X |
| 3 | Phenols | Ferric chloride test | √ | √ | √ | √ | X | X | X | √ | √ | X |
| 4 | Saponins | Foam test | X | √ | √ | √ | X | X | X | X | √ | X |
| 5 | Steroids and Terpenoids | Liebermann-Burchard's test | X | X | X | X | √ | √ | X | X | √ | X |
| 6 | Phytosterols | Salkowski's test | X | X | √ | X | √ | √ | X | X | X | X |
| 7 | Flavonoids | Alkaline reagent test | √ | √ | √ | √ | X | X | X | X | X | √ |
| 8 | Amino acids and Proteins | Ninhydrin test | √ | X | √ | √ | X | X | X | X | X | √ |
| 9 | Carbohydrates | Molisch's test | √ | X | √ | √ | X | X | X | √ | √ | X |
| | | Fehling's test | √ | √ | √ | √ | X | X | X | √ | √ | X |

A: Hexane extract; B: Chloroform extract; C: Ethyl acetate extract; D: Alcohol extract; E: Aqueous extract; F: Hydroalcoholic extract. 'X' indicates not detected; '√' indicates detected.

antileishmaniasis agents[65], and 3-β-hydroxy-2,3-dihydro-withanolide was reported to be neuroprotective[66]. Withanamide (A-H) was shown to help neutralize the toxicity of β-amyloid protein and protect against cell death in Alzheimer's disease. It also inhibited lipid peroxidation[67]. The compound 4β, 20-dihydroxy-i-oxo-5β,6β, epoxy-witha-2,24-dienolide was found to have antitumor activity. Withanolide-E had antifeedant activity[43]. Sitoindosides IX and X were responsible for the immunomodulatory activity of the *Withania* species. Bioactive constituents present in *Withania* such as withanosides, sitoindosides, steroidal lactones, and alkaloids were found to have a broad spectrum of therapeutic potential[35].

## IMMUNOMODULATORY ACTION AGAINST NOVEL CORONAVIRUS

The importance of medicinally important natural products has increased because of the coronavirus disease 2019 (COVID-19) pandemic and its aftermath[68-71]. The lack of an effective medicine or vaccines led investigators to search for natural products that are easily available and can help fight this disease either by being viricidal or by strengthening the immune system. Proper understanding of the interaction between the coronavirus and cell-surface receptors forms the basis of developing curative medications to combat novel coronavirus. Several medicinal plants have been reported to

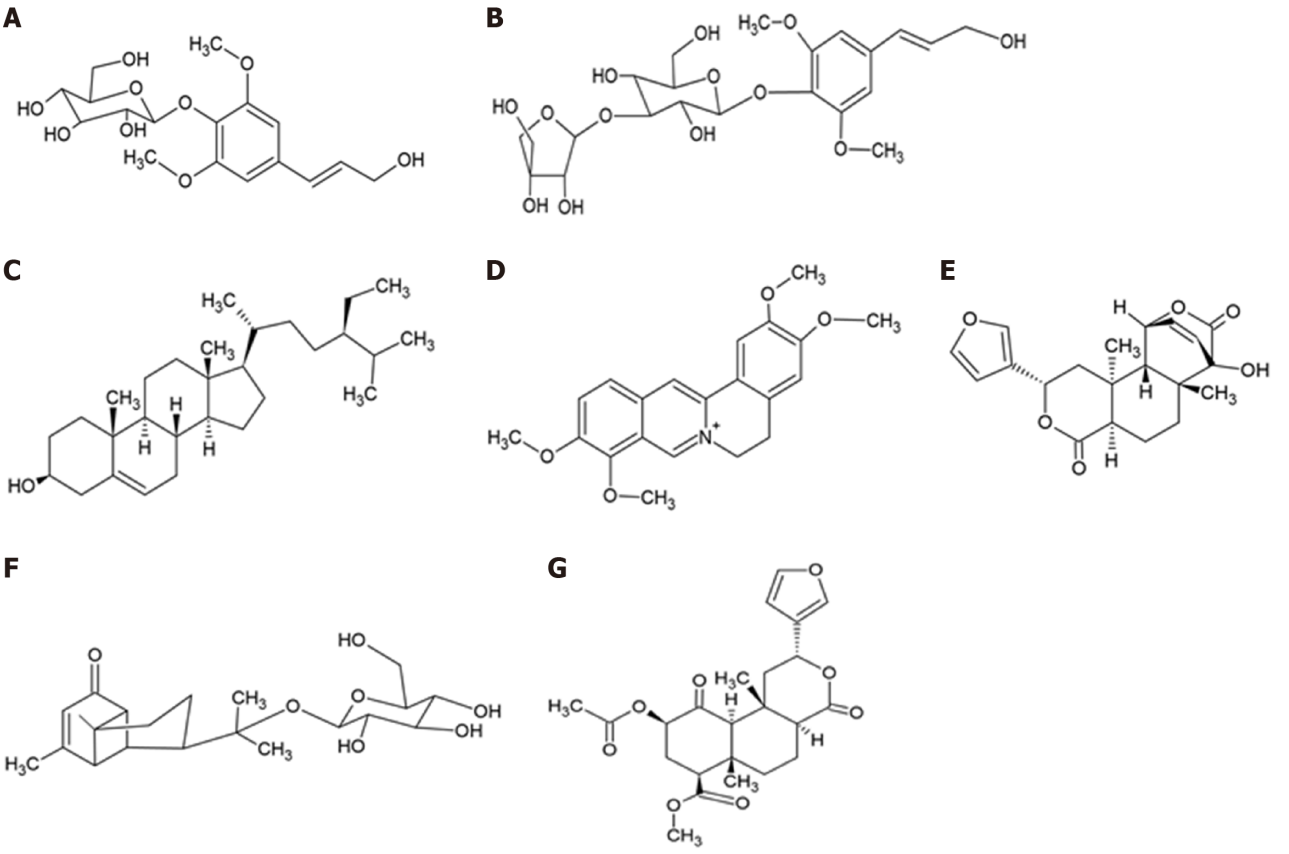


**Figure 1 Important compounds isolated from *Tinospora cordifolia*.** A: Syringin; B: Cordiofolioside A; C: β-sitosterol; D: Palmatine; E: Columbin; F: Tinocordiside; G: Furanolactone.

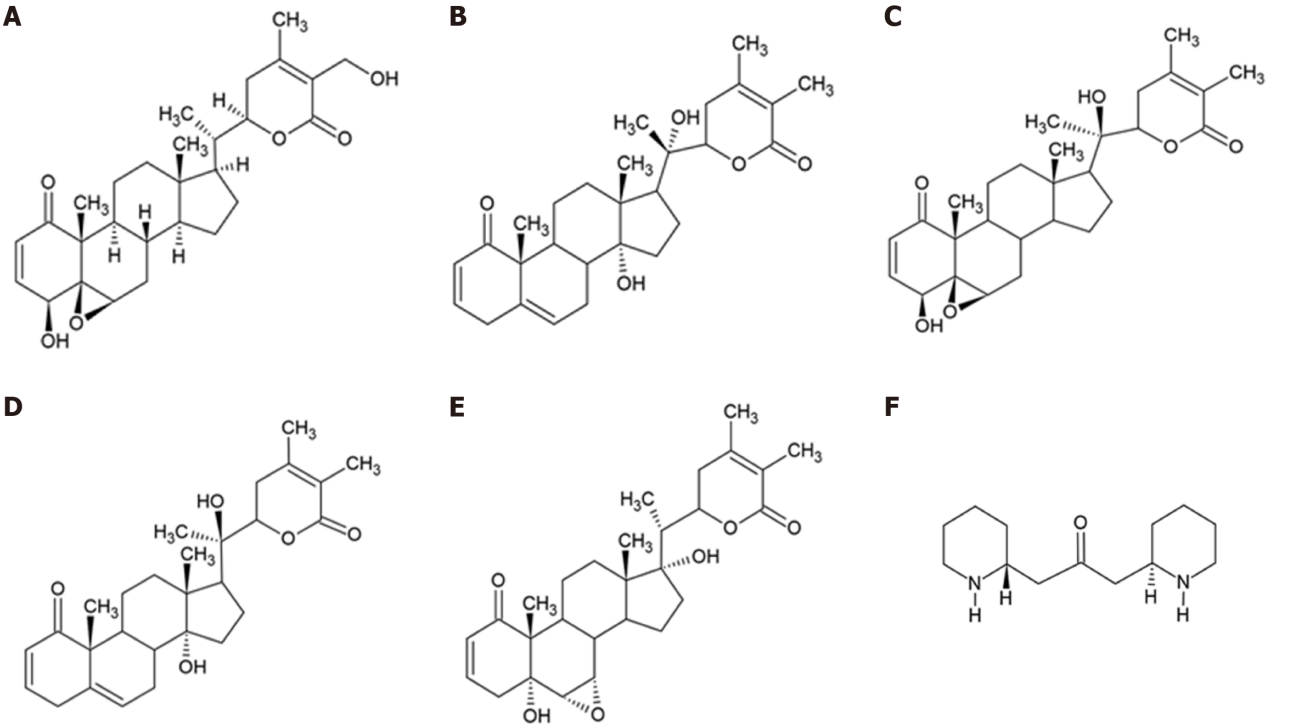


**Figure 2 Important compounds isolated from *Withania somnifera*.** A: Withaferin A; B: Withanolide A; C: Withanolide D; D: Withanolide G; E: Withanone; F: Anaferine.

produce pharmacologically active compounds that have effective antiviral properties[1,72]. This study included natural compounds that reduce the risk of coronavirus infection by inhibiting viral entry into the host cell. *W. somnifera* and *T. cordifolia* have proven to be among the most important plants in combating novel coronavirus. Computational studies have been carried out to determine the efficacy of active compounds obtained from both plants against coronavirus[30].

Studies of the phytochemicals of *W. somnifera* have shown them to have great potential as antiviral agents for treating viral diseases, including human papillomavirus, H1N1 influenza, *Herpes simplex*, SARS-CoV, and SARS-CoV-2. The Indian Government, the Indian Council of Medical Research, and the Council of Scientific and Industrial Research have issued advisories emphasizing the use of *W. somnifera* as a therapeutic agent against COVID-19[64,73-76]. As mentioned previously, withanolide is the main active constituent of *W. somnifera*. Withanolide D, withaferin A, and withanoside I-VII were reported to have significant and effective biological activity in managing COVID-19. Molecular docking studies have shown that natural ingredients of *W. somnifera* namely, withanone, caffeic acid phenyl ester, and some other biologically active substances interact with novel coronavirus receptors and impede interaction of the virus with host cells [77,78]. In a docking study by Borse *et al*[70], ashwagandhanolide had the highest docking score (−9.9 Kcal/mol) for all three SARS-CoV-2 protein targets having its drug-likeness. Molecular docking results reported by Prajapati *et al*[79] showed that flavone glycoside, sugar alcohol, and flavonoid present in *W. somnifera* had binding potentials of −11.69, −11.61, −10.1, and −7.71 kcal/mol, respectively, for the spike-protein on the surface of coronavirus, CD26, RdRp, and TMPRSS2 proteins.

Twenty-eight important phytochemicals are present in *T. cordifolia*, including tinocordiside, an active ingredient with a strong binding affinity for the novel coronavirus. This phytochemical has proven to be an effective immunomodulator and has been found to inhibit the infection of host cells by coronavirus[50,80-85]. Docking analysis and its absorption, distribution, metabolism, excretion, and toxicity revealed that six of thirty-one potential constituents (alkaloids, steroids, and terpenoids) of a *T. cordifolia* extract had strong interactions with human SARS-CoV-2 receptors, prevented the entry of the virus, and thus have potential for COVID-19 prophylaxis[86]. A ketone, tinosponone from *T. cordifolia* is a strong inhibitor of the 3CL major protease of SARS-CoV-2, as shown by computer-aided drug design. Confirmation of its inhibitory activity on SARS-CoV-2 needs to be shown in *in vitro* and *in vivo* studies[87]. *In silico* studies revealed that saponarin, a phytochemical present in *T. cordifolia*, had a binding affinity of −8.75 kcal/mol and a was a potential inhibitor of the main protease of COVID-19[86-89]. Thakkar *et al*[90] reported that columbin, tinosporide, N-trans-feruloyl-tyramine-diacetate, amritoside C, amritoside B, amritoside A, tinocordifolin, palmatoside G, palmatoside F, and maslinic acids were key molecules for further study based on their docking scores, which ranged from −5.02 to −5.72 Kcal/mol)[88,90]. *T. cordifolia* has been linked to autoimmune acute hepatitis and a study found that its use can reveal autoimmune hepatitis in patients[91]. In the interest of public health, further research is needed on the safety and effectiveness of unproven but widely marketed herbal remedies in alternative medical systems, and it is particularly important in light of the current worldwide health crisis[91,92].

## CONCLUSION

This review has discussed evidence that both *T. cordifolia* and *W. somnifera* hold a special place in the ancient texts and they have proven to be equally important in modern medicine. Extraction and phytochemical analysis have revealed that these plants are rich sources of numerous biologically active compounds. Since the COVID-19 pandemic outbreak, a lot of attention has been directed toward increasing the immunity of the human body against various pathogens such as bacteria, viruses, *etc.* The immunomodulatory activities of *T. cordifolia* and *W. somnifera* have been a part of Ayurvedic medication for a long time. Recent clinical studies of the products obtained from these plants have confirmed their therapeutic action in benefiting the immune system.

While the preliminary findings are promising, further clinical trials and research are needed to establish standard dosages and the safety and efficacy of these phytochemicals in the context of COVID-19. Integration of these herbal remedies with conventional treatment would offer a holistic approach to managing the disease, improving patient outcomes, and enhancing overall health resilience. In conclusion, *T. cordifolia* and *W. somnifera* have significant therapeutic potential, particularly as supportive treatment of COVID-19. Their rich phytochemical composition and multifaceted health benefits underscore the importance of further research and validation in clinical settings.

## ACKNOWLEDGEMENTS

The authors are thankful to Amity University Uttar Pradesh, India, for providing the necessary infrastructure and library facility for the compilation of information.

## FOOTNOTES

**Author contributions:** Gupta D contributed to conceptualization, review, editing, and supervision; Rai P and Garain T contributed to original draft preparation, literature search, resources, and writing; All authors reviewed the successive versions of the manuscript and read and agreed to the last version of the manuscript for publication. Rai P and Garain T contributed equally in their efforts as outlined above and their collaboration was crucial to this paper's generation and publication, and as such they merit co-first authorship.

**Conflict-of-interest statement:** No competing interests or conflicts to declare.



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**S-Editor:** Liu JH
**L-Editor:** Filipodia
**P-Editor:** Zheng XM

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World Journal of Experimental Medicine

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*World J Exp Med* 2024 September 20; 14(3): 96269

DOI: 10.5493/wjem.v14.i3.96269

ISSN 2220-315x (online)

*MINIREVIEWS*

# Aryl hydrocarbon receptor dynamics in esophageal squamous cell carcinoma: From immune modulation to therapeutic opportunities

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**Specialty type:** Immunology

**Provenance and peer review:** Invited article; Externally peer reviewed.

**Peer-review model:** Single blind

**Peer-review report's classification**

**Scientific Quality:** Grade B
**Novelty:** Grade A
**Creativity or Innovation:** Grade A
**Scientific Significance:** Grade B

**P-Reviewer:** Wang Z

**Received:** May 1, 2024
**Revised:** May 26, 2024
**Accepted:** June 14, 2024
**Published online:** September 20, 2024
**Processing time:** 119 Days and 20.8 Hours

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## Abstract

Esophageal squamous cell carcinoma (ESCC) is a substantial global health burden. Immune escape mechanisms are important in ESCC progression, enabling cancer cells to escape the surveillance of the host immune system. One key player in this process is the Aryl Hydrocarbon Receptor (AhR), which influences multiple cellular processes, including proliferation, differentiation, metabolism, and immune regulation. Dysregulated AhR signaling participates in ESCC development by stimulating carcinogenesis, epithelial-mesenchymal transition, and immune escape. Targeting AhR signaling is a potential therapeutic approach for ESCC, with AhR ligands showing efficacy in preclinical studies. Additionally, modification of AhR ligands and combination therapies present new opportunities for therapeutic intervention. This review aims to address the knowledge gap related to the role of AhR signaling in ESCC pathogenesis and immune escape.

**Key Words:** Esophageal squamous cell carcinoma; Aryl hydrocarbon receptor; Immune escape; Tumor microenvironment; Immunosuppression; Therapeutic targeting

**Core Tip:** Esophageal squamous cell carcinoma (ESCC) utilizes immune escape mechanisms, including major histocompatibility complex downregulation and immune checkpoint manipulation, enhancing tumor progression. The aryl hydrocarbon receptor (AhR), crucial in health and disease, significantly influences ESCC development and immune evasion through carcinogenic pathways. AhR activation triggers proliferation, inhibits apoptosis, and induces epithelial-mesenchymal transition. Moreover, AhR suppresses effector T cells and enrolls immunosuppressive myeloid cells. Therapeutic AhR targeting with AhR ligands showed promise in inhibiting tumor growth, controlling the immune microenvironment, and enhancing treatment efficacy. Combining AhR-targeted therapies with conventional treatments or dietary interventions has the potential to improve ESCC patient outcomes.

**Citation:** Rahmati M, Moghtaderi H, Mohammadi S, Al-Harrasi A. Aryl hydrocarbon receptor dynamics in esophageal squamous cell carcinoma: From immune modulation to therapeutic opportunities. *World J Exp Med* 2024; 14(3): 96269
**URL:** https://www.wjgnet.com/2220-315x/full/v14/i3/96269.htm
**DOI:** https://dx.doi.org/10.5493/wjem.v14.i3.96269

## INTRODUCTION

Esophageal squamous cell carcinoma (ESCC), a severe malignancy of the esophagus, shows a significant global health burden (ESCC is the eighth most common cancer globally)[1]. It shows a notable geographical variation, with particularly high incidence rates in East Asia, Central Asia, and certain regions of Africa[2].

ESCC emerges through a multistep process involving progressive genetic and molecular alterations within the esophageal epithelium[3]. Chronic exposure to established carcinogens, such as tobacco smoke and excessive alcohol consumption, triggers cellular damage and initiates a cascade of events leading to uncontrolled proliferation and malignant transformation[4]. These factors can lead to point mutations in tumor suppressor genes such as p53, which regulate cell cycle progression and DNA repair[5]. Moreover, amplification of oncogenes, such as cyclin D1, promote cell cycle dysregulation and uncontrolled growth[6]. Furthermore, epigenetic modifications, such as DNA methylation and histone acetylation configurations, can inhibit tumor suppressor genes and provide a pro-tumorigenic microenvironment[7]. These genetic and molecular aberrations interfere with normal cellular processes, leading to the development of pre-cancerous lesions characterized by squamous epithelial hyperplasia and dysplasia. If left unchecked, these pre-cancerous lesions may progress to invasive ESCC.

Despite advancements in treatment procedures, ESCC prognosis is still poor, emphasizing the need for a better understanding of the main processes that stimulate tumorigenesis and immune escape.

## IMMUNE ESCAPE: A CANCEROUS DEFENSE MECHANISM

ESCC development is counterbalanced by the host immune system, which employs a two-edged plan for immune surveillance, including innate and adaptive immunity[8]. The ability of cancer cells to evade the detection of immune system and elimination is a hallmark of tumor progression[9]. In ESCC, the immune escape demonstrates through a sophisticated connection of various mechanisms utilized by tumor cells to create a microenvironment that protects them from immune attack[10].

To escape the strong immune surveillance system, ESCC cells organize a multi-dimensional immune escape program (Figure 1). One of the key mechanisms includes downregulation of major histocompatibility complex (MHC) molecules on the cell surface[11]. MHC molecules function as antigen presentation platforms, allowing T cells to recognize and eliminate foreign or abnormal cells. By reducing MHC expression, ESCC cells become undetectable to the immune system, making them resistant to T cell-mediated cytotoxicity[12].

Furthermore, ESCC cells actively modulate the tumor microenvironment to create an immunosuppressive state[13]. They achieve this by secreting chemokines and cytokines that recruit and activate regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs)[10]. The suppressive cell populations directly inhibit the T lymphocytes (CTLs) and suppress the immune response[8].

Immune escape in ESCC also involves utilization of immune checkpoint pathways. These pathways, mediated by molecules such as PD-L1 and CTLA-4, act as a regulatory brake on the immune system to prevent excessive immune activation and autoimmunity[13,14]. ESCC cells can upregulate the expression of these checkpoint ligands, allowing them to bind to their corresponding receptors on T cells and effectively deactivate the anti-tumor T cell response[15].

In addition to the previously mentioned mechanisms, ESCC cells show a flexible immune escape repertoire. They can express ligands that bind to inhibitory receptors on T cells, leading to T cell exhaustion and dysfunction[13]. ESCC cells can also reprogram their metabolism to escape immune recognition and resist immune-mediated cytotoxicity[10]. Furthermore, they can produce immunosuppressive enzymes, such as indoleamine 2,3-dioxygenase, which reduces essential amino acids required for T cell activation and function, disabling the anti-tumor immune response[16].

By using these diverse immune escape methods, ESCC cells create a microenvironment that protects them from immune attack and promotes tumor progression. Understanding these mechanisms is crucial for developing novel therapeutic strategies that can enhance the anti-tumor immune response and improve patient outcomes.

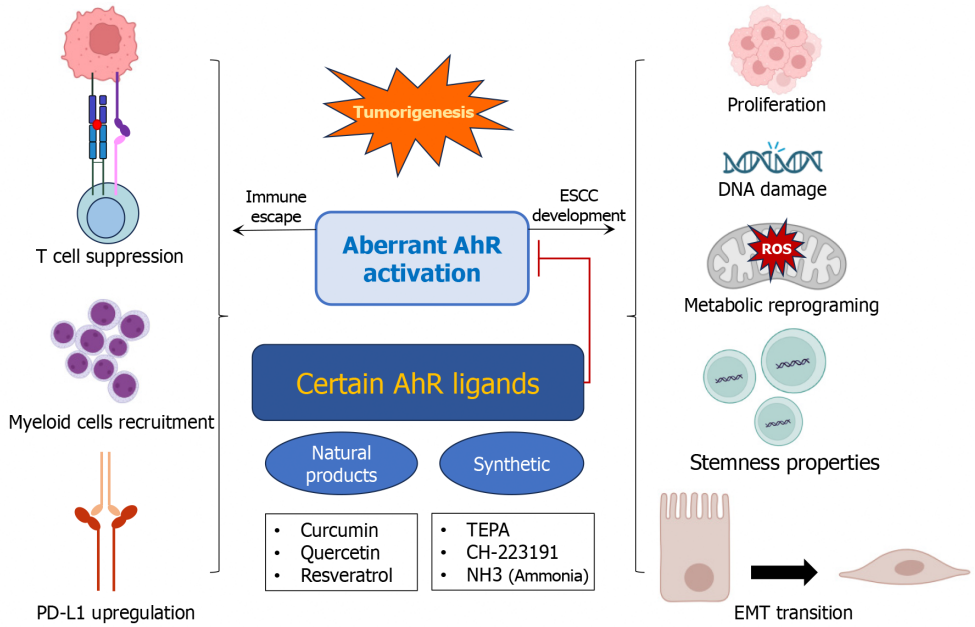


**Figure 1 Aryl hydrocarbon receptor signaling in esophageal squamous cell carcinoma development and immune escape.** This figure illustrates the multifaceted influence of aryl hydrocarbon receptor (AhR) signaling on esophageal squamous cell carcinoma (ESCC) development and immune escape. Activation of AhR by environmental carcinogens or other ligands initiates a cascade of cellular processes that contribute to tumorigenesis. EMT: Epithelial-mesenchymal transition; NH3: Ammonia; PD-L1: Programmed death-ligand 1; ROS: Reactive oxygen species; TEPA: Triethylenetetramine.

## ARYL HYDROCARBON RECEPTOR SIGNALING: A MASTER REGULATOR WITH DIVERSE FUNCTIONS IN HEALTH AND DISEASE

Aryl hydrocarbon receptor (AhR) is a ubiquitous and complicated ligand-activated transcription factor that has significant influences on various physiological processes[17]. AhR, which is primarily found in the cytoplasm with chaperone proteins, goes through significant changes upon binding to ligands[18]. These ligands are categorized into two main groups: Exogenous, which includes environmental pollutants such as polycyclic aromatic hydrocarbons (PAHs), and endogenous, which comprises metabolites from tryptophan breakdown, such as tryptophan and kynurenine[19]. When a ligand binds, AhR alters its shape, releases from its chaperone complex, and moves to the nucleus[20]. It pairs with ARNT and attaches to specific DNA sequences called xenobiotic response elements in the regulatory regions of the target genes. This interaction initiates the transcription of various genes, influencing several cellular processes *via* AhR signaling[21]. Beyond its well-recognized role in xenobiotic metabolism, AhR signaling applies an excessive influence on numerous biological processes, including cellular proliferation and differentiation[22], angiogenesis[23], cellular metabolism[24], oxidative stress response[25], immune regulation[26], and inflammation[27].

Studies have demonstrated that AhR signaling affects the proliferation and differentiation of epithelial cells, lymphocytes, and hematopoietic progenitor cells[22]. Moreover, AhR may influence the expression of pro-angiogenic factors such as vascular endothelial growth factor, and impact both physiological and pathological angiogenesis[23]. AhR activation also influences insulin sensitivity, glucose uptake, and lipogenesis. Dysregulation of these pathways may contribute to the development of metabolic disorders, such as obesity and type 2 diabetes[24]. AhR signaling can influence cellular metabolism and, promote the Warburg effect. This metabolic shift not only enhances tumor cell proliferation, but can also create an immunosuppressive microenvironment by changing the levels of metabolites and signaling molecules that modulate immune cells[28]. The cellular environment is consistently influenced by the reactive oxygen species produced during normal metabolic processes. AhR can modulate the antioxidant defense system by targeting the expression of enzymes such as heme oxygenase-1, NAD, and quinine oxidoreductase 1, which are important in protecting cells from oxidative damage[25].

AhR signaling exerts a detailed and context-specific influence on the immune system. It modulates the function of several immune cell populations, including T cells, B cells, and antigen-presenting cells (APCs)[26]. AhR activation can promote the differentiation of Tregs with immunosuppressive properties, while simultaneously inhibiting the proliferation and function of effector T cells[29]. AhR can influence the expression of MHC, and co-stimulatory molecules on APCs, and impact the efficiency of antigen presentation and T cell activation[30]. The tumor microenvironment is often characterized by chronic inflammation, which can promote tumorigenesis. AhR signaling can influence the expression of inflammatory mediators, such as cytokines and chemokines, forming an inflammatory milieu within the tumor microenvironment. While some inflammatory responses can promote anti-tumor immunity, chronic inflammation initiated by aberrant AhR activation can create a pro-tumorigenic microenvironment[27]. The multi-dimensional nature of the AhR highlights its potential as a double-edged sword. While it contributes to essential physiological processes, including xenobiotic metabolism, immune homeostasis, and tissue development, its dysregulation can be involved in various disease pathologies, including cancer. Understanding the important role of AhR signaling in ESCC, especially its

influence on the tumor immune microenvironment and immune escape mechanisms, could be beneficial in the development of novel therapeutic strategies.

## AHR AND ESCC DEVELOPMENT

Exposure to environmental carcinogens, such as PAHs found in cigarette smoke and certain dietary components is a well-established risk factor for ESCC development[31]. These carcinogens can activate AhR signaling *via* direct ligand binding [32]. The activated AhR pathway influences multiple cellular processes that contribute to ESCC tumorigenesis. Studies have demonstrated that AhR activation can promote the proliferation of esophageal epithelial cells by modulating cell cycle regulatory proteins such as cyclin D1 and p21[33]. Moreover, AhR signaling can inhibit apoptosis and, the programmed cell death pathway, by influencing the expression of anti-apoptotic factors such as Bcl-2[34]. These effects create conditions favorable for deregulated cell proliferation, which is a characteristic of cancer. Chronic exposure to carcinogens can induce DNA damage in esophageal epithelial cells[35]. However, AhR activation can further exacerbate this issue by downregulating the expression of key DNA repair enzymes[36]. This impaired repair system increases the accumulation of mutations and, eventually increases the risk of malignant transformation. Cancer cells exhibit a distinct metabolic profile compared to healthy cells. ESCC development and progression may be initiated by a subpopulation of cancer stem cells with self-renewal and differentiation capabilities[37,38]. AhR activation has been studied in promoting the stemness properties of ESCC cells[39].

Epithelial-mesenchymal transition (EMT) is a critical process by which epithelial cells lose their polarized phenotype and acquire mesenchymal characteristics, gaining migratory and invasive potential[40]. AhR activation has been shown to induce EMT in various cell types, including esophageal epithelial cells[41]. Studies have demonstrated that AhR ligands can upregulate the expression of EMT-inducing transcription factors such as Twist1 and Snail, promoting the acquisition of an invasive phenotype by cancer cells[42]. The EMT process is crucial for ESCC progression and metastasis, by enabling cancer cells to detach from the primary tumor, migrate through the basement membrane, and invade the surrounding tissues and lymphatic vessels. EMT can also enhance the resistance of ESCC cells to anoikis, a type of cell death triggered by detachment from the extracellular matrix[43]. This combined effect of increased motility and resistance to anoikis promotes the dissemination of ESCC cells to other organs, resulting in the formation of metastases.

## AHR AND IMMUNE ESCAPE IN ESCC

While certain studies have indicated that activating AhR might enhance anti-cancer immune responses, other studies suggest its possible involvement in immune evasion mechanisms in ESCC. AhR activation can suppress effector T cells, the main attacking cells of the adaptive immune response[44]. Studies have shown that some AhR ligands can inhibit the proliferation and cytokine production of CD8+ CTLs and weaken the immune response against ESCC cells[45]. Additionally, AhR activation can promote the differentiation of Tregs, a population of immunosuppressive T cells that dampens the anti-tumor immune response[46]. The tumor microenvironment in ESCC is often infiltrated by immunosuppressive myeloid cells, including MDSCs[13]. AhR activation promotes the recruitment and expansion of MDSCs, which contributes to the suppression of anti-tumor immunity[47]. Immune checkpoint molecules, such as PD-L1 and CTLA-4, act as inhibitory agents in the immune system to prevent autoimmunity. However, ESCC cells can use these checkpoints to escape immune response[48]. Some studies suggested that AhR activation upregulates the expression of PD-L1 on ESCC cells, potentially participating in immune escape by inhibiting T cell function[49]. Table 1 summarizes the influence of AhR signaling on ESCC development, progression, and immune escape.

## THERAPEUTIC POTENTIAL OF TARGETING AHR SIGNALING IN ESCC

The complex influence of AhR signaling on ESCC development, progression, and immune escape presents an interesting opportunity for the development of novel therapeutic strategies. While the aberrant AhR activation can promote tumorigenesis and create an immunosuppressive microenvironment, its proper modulation could be beneficial for disrupting these processes and boosting the anti-tumor immune response.

Preclinical studies investigating AhR ligands in ESCC models have yielded in interesting results[49]. Several antagonists could competitively bind to the ligand-binding domain, and block the activation of AhR signaling pathways and their downstream effects on ESCC cells[49]. Studies have demonstrated that AhR antagonists can suppress ESCC cell proliferation, migration, and invasion, potentially inhibiting tumor progression and metastasis[50]. AhR antagonists exhibit immunomodulatory effects within the tumor microenvironment, by inhibiting AhR signaling. These antagonists may reduce the population of immunosuppressive MDSCs and Tregs, and simultaneously enhancing the function and proliferation of effector T cells, leading to a more powerful anti-tumor immune response[51,52]. Several AhR antagonists are currently under investigation for their therapeutic potential in ESCC, including CH-223191, NH3 (Ammonia), and specific small molecules[53]. Not only AhR antagonists but also some naturally derived AhR agonists, such as curcumin [54] and quercetin[55] have been shown to exhibit regulatory properties on immune response in cancer. This controversy could be due to the structural and metabolic differences of different AhR ligands. Furthermore, synthetic AhR antagonists are being actively developed, with some demonstrating promising preclinical results[51]. Table 2 has summarized some

**Table 1 Aryl hydrocarbon receptor signaling and its impact on**

| Aspect of ESCC | Impact of AhR activation | Mechanism | Ref. |
|---|---|---|---|
| Carcinogenesis | | | |
| Proliferation | Increased | Upregulates cyclin D1, downregulates p21 | [31,33] |
| Apoptosis | Inhibited | Upregulates anti-apoptotic factors like Bcl-2 | [34] |
| DNA repair | Impaired | Downregulates expression of key DNA repair enzymes | [36] |
| Metabolic reprogramming | Promotes Warburg effect | Increases reliance on aerobic glycolysis | [28] |
| Stemness | Enhanced | Promotes self-renewal and differentiation of cancer stem cells | [37,38] |
| EMT | Induced | Upregulates EMT-inducing transcription factors like Twist1 and Snail | [41,43] |
| Immune escape | | | |
| T cell function | Suppressed | Inhibits proliferation and cytokine production of CD8+ CTLs, promotes differentiation of Tregs | [45,46] |
| Myeloid cell recruitment | Increased | Promotes recruitment and expansion of MDSCs | [47] |
| Immune checkpoint regulation | Potential upregulation of PD-L1 expression | May contribute to immune escape by inhibiting T cell activity | [49] |

AhR: Aryl hydrocarbon receptor; Bcl-2: B-cell lymphoma 2; CD8+ CTLs: CD8+ Cytotoxic T lymphocytes; EMT: Epithelial-mesenchymal transition; ESCC: Esophageal squamous cell carcinoma; MDSCs: Myeloid-derived suppressor cells; PD-L1: Programmed death-ligand 1; Snail: Zinc finger protein SNAI1; Twist1: Twist family BHLH Transcription factor 1; Tregs: Regulatory T cells.

**Table 2 Mechanisms of action and potential effects of promising aryl hydrocarbon receptor antagonists on malignancies**

| AhR ligands | Mechanism of action | Potential effects on malignancies | Ref. |
|---|---|---|---|
| Natural products | | | |
| Curcumin | Competitive binding to the AhR ligand-binding domain | Suppresses proliferation, migration, and invasion of cancer cells; may enhance anti-tumor immunity | [54,62,63] |
| Quercetin | Competitive binding to the AhR ligand-binding domain; may also inhibit AhR nuclear translocation | Suppresses proliferation and induces apoptosis in cancer cells | [55] |
| Resveratrol | May interfere with AhR-DNA binding; may also possess antioxidant and anti-inflammatory properties | May inhibit tumor growth and metastasis | [55,64] |
| Synthetic ligands | | | |
| CH-223191 | Competitive binding to the AhR ligand-binding domain | Suppresses proliferation and migration of cancer cells | [65] |
| NH3 (Ammonia) | Competitive binding to the AhR ligand-binding domain | Shows promise in preclinical models, but may have limitations due to potential toxicity | [66] |
| Small molecule antagonists | Competitive binding to the AhR ligand-binding domain or interfering with AhR dimerization | Several novel small molecules are under development, with preclinical studies ongoing | [67,68] |

AhR: Aryl hydrocarbon receptor.

of the most promising candidates, their mechanisms of action, and potential effects on malignancies.

Beyond directly targeting the AhR itself, manipulating the endogenous ligands that activate this pathway offers another potential therapeutic avenue. The gut microbiota plays an important role in metabolizing dietary components into ligands that can activate AhR signaling[56]. Strategies aimed at modulating the gut microbiota composition, potentially through prebiotics or probiotics, could be studied to influence the production of these ligands and their subsequent impact on AhR signaling in ESCC[57]. Moreover, dietary factors can also influence AhR ligand production. Identifying and employing natural AhR modulators, such as indole-3-carbinol found in cruciferous vegetables, into the diet could be a preventive or complementary therapeutic approach for ESCC patients[58,59]. Some AhR agonists such as TCDD bind tightly to the AhR, leading to long-lasting activation that disrupts normal cellular processes and promotes cancer development. However, I3C, and its metabolites transiently activate the AhR. This controlled activation helps in detoxifying carcinogens, inhibiting abnormal cell growth, and promoting cancer cell death, highlighting their potential anticancer effects[58,59]. However, the effectiveness of dietary AhR modulators is not well-established by the lack of

comprehensive studies on the metabolic kinetics, permeability, and transport of dietary compounds. Future research should focus on these aspects to better understand and optimize the role of these compounds on ESCC treatment.

The therapeutic potential of AhR-targeted strategies may be further intensified when combined with existing treatment approaches for ESCC. For instance, combining selected AhR ligands (agonist or antagonist) with conventional therapies such as surgery, radiation, or chemotherapy could offer synergistic effects. These traditional therapies can induce DNA damage and cell death in ESCC cells, while AhR ligands may suppress tumor growth and metastasis by inhibiting proliferation and invasion[60]. Additionally, AhR ligands could be particularly effective when combined with immune checkpoint inhibitors[61]. Clinical trials investigating the combination of AhR-targeted therapies with other treatment strategies are suggested to determine their efficacy and safety in ESCC patients.

## CONCLUSION

Immune escape mechanisms play a crucial role in ESCC progression, allowing cancer cells to evade the surveillance of the host immune system. The AhR activation, triggered by environmental carcinogens such as PAHs, promotes various hallmarks of cancer, including proliferation, apoptosis inhibition, and metabolic reprogramming. Moreover, AhR signaling contributes to immune escape by suppressing T cell function, recruiting immunosuppressive myeloid cells, and upregulating immune checkpoint molecules like PD-L1. Targeting AhR signaling presents a promising therapeutic approach for ESCC. AhR ligands (agonist or antagonist), both natural and synthetic, have shown potential in preclinical studies by inhibiting tumor growth and modulating the immune microenvironment. Modulating AhR ligands through dietary interventions may offer new therapeutic approaches. Furthermore, combining AhR-targeted therapies with conventional treatments or immune checkpoint inhibitors may result in synergistic effects, enhancing overall therapeutic efficacy.

## FOOTNOTES

**Author contributions:** Rahmati M and Moghtaderi H reviewed the literature, prepared information, and drafted the manuscript; Mohammadi S and Al-Harrasi A conceptualized the review, proposed the title, and provided critical reviews; All authors have read and approved the final manuscript. Rahmati M and Moghtaderi H reviewed relevant literature, gathered essential information, and drafted the initial version of the manuscript. Rahmati M organized the data and ensured the integration of comprehensive and relevant studies into the review. Moghtaderi H synthesized the gathered information and contributed significantly to the initial manuscript draft. Mohammadi S and Al-Harrasi A conceptualized the overall scope of the review article and proposed the title. Mohammadi S offered essential perspectives that maintained the manuscript's logical flow and improved its scientific precision during the revision stages. Al-Harrasi A conducted extensive reviews and contributed to revising the content to meet high academic standards. Both Rahmati M and Moghtaderi H made crucial contributions towards the completion of the review, qualifying as co-first authors. Both Mohammadi S and Al-Harrasi A played important and essential roles in the conceptual design, critical review, and manuscript preparation as co-corresponding authors. Mohammadi S supervised the entire review process, provided continuous oversight, and ensured the manuscript's coherence. Al-Harrasi A contributed significantly to the critical review, enhancing the manuscript's quality and accuracy. This collaboration between Mohammadi S and Al-Harrasi A was essential for the successful completion and publication of this manuscript. Their combined efforts in conceptual design, critical review, and supervision were crucial for maintaining the manuscript's high standards and academic integrity.

**Conflict-of-interest statement:** All the authors declare that there are no conflicts of interest to disclose.



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**S-Editor:** Liu JH
**L-Editor:** Filipodia
**P-Editor:** Wang WB

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*World J Exp Med* 2024 September 20; 14(3): 97233

DOI: 10.5493/wjem.v14.i3.97233

ISSN 2220-315x (online)

*MINIREVIEWS*

# Anti-aging based on stem cell therapy: A scoping review

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**Specialty type:** Medicine, research and experimental

**Provenance and peer review:** Unsolicited article; Externally peer reviewed.

**Peer-review model:** Single blind

**Peer-review report's classification**

**Scientific Quality:** Grade C
**Novelty:** Grade B
**Creativity or Innovation:** Grade C
**Scientific Significance:** Grade C

**P-Reviewer:** Bonartsev A

**Received:** May 26, 2024
**Revised:** July 4, 2024
**Accepted:** July 18, 2024
**Published online:** September 20, 2024
**Processing time:** 94 Days and 16.8 Hours

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## Abstract

Stem cells are present in the tissues and organs and remain in a quiescent and undifferentiated state until it is physiologically necessary to produce new descendant cells. Due to their multipotency property, mesenchymal stem cells have attracted considerable attention worldwide due to their immunomodulation and therapeutic function in tissue regeneration. Stem cells secrete components such as paracrine factors, extracellular vesicles, and exosomes which have been shown to have anti-inflammatory, anti-aging, reconstruction and wound healing potentials in many *in vitro* and *in vivo* models. The pluripotency and immunomodulatory features of stem cells could potentially be an effective tool in cell therapy and tissue repair. Aging affects the capacity for self-renewal and differentiation of stem cells, decreasing the potential for regeneration and the loss of optimal functions in organisms over time. Current progress in the field of cellular therapy and regenerative medicine has facilitated the evolution of particular guidelines

and quality control approaches, which eventually lead to clinical trials. Cell therapy could potentially be one of the most promising therapies to control aging due to the fact that single stem cell transplantation can regenerate or substitute the injured tissue. To understand the involvement of stem cells not only in tissue maintenance and disease but also in the control of aging it is important to know and identify their properties, functions, and regulation *in vivo*, which are addressed in this review.

**Key Words:** Mesenchymal stem cell; Anti-aging; Telomerase; Regenerative medicine; Stem cell therapy



**Core Tip:** Mesenchymal stem cells (MSCs) exhibit promising anti-aging properties by targeting the underlying mechanisms of aging, including chronic inflammation, cellular senescence, and oxidative stress. Through their regenerative potential, immunomodulatory abilities, and secretome production, MSCs contribute to tissue repair and rejuvenation. These unique capabilities position MSCs as key players in the future of anti-aging therapies and regenerative medicine, offering potential interventions for promoting healthier and more vibrant aging.

**Citation:** El Assaad N, Chebly A, Salame R, Achkar R, Bou Atme N, Akouch K, Rafoul P, Hanna C, Abou Zeid S, Ghosn M, Khalil C. Anti-aging based on stem cell therapy: A scoping review. *World J Exp Med* 2024; 14(3): 97233
**URL:** https://www.wjgnet.com/2220-315x/full/v14/i3/97233.htm
**DOI:** https://dx.doi.org/10.5493/wjem.v14.i3.97233

## INTRODUCTION

Aging is the time-related deterioration of essential physiological functions for survival and fertility, occurring universally in various organisms and leading to natural death. This process involves gradual dysfunctions in all organs, affecting unicellular organisms, plants, animals, and humans alike. It is an inevitable stage of development and life. Zhang *et al*[1] define aging as the reduction of the body's capacity, both physically and psychologically, to adapt to the environment, and the gradual tendency towards death.

Recent gerontological research has delved into the complex biochemical mechanisms associated with the gradual decline in bodily functions. It emphasizes the involvement of multiple processes at the cellular, molecular, and systemic level, requiring comprehensive approaches for understanding aging. Despite being a physiological and universal process, aging occurs at an individual rate in each person[2,3].

Differentiating between chronological and biological age is important due to the varying rates of aging. Chronological age merely indicates the time passed since birth, while biological age encompasses a broad spectrum of physical, physiological, and cognitive functions, influenced by molecular and cellular processes[4]. Despite ongoing discussions, the biology of aging remains controversial, posing challenges in establishing a universally accepted definition of normal aging[5]. However, biological aging relies on various theories, including those related to the effects of free radicals, telomere shortening, and the mitochondrial theory.

In the broader context of anti-aging research, healthy aging is characterized by the attainment of an extended period of robust physiological and mental well-being. This entails an ongoing effort to seize opportunities for enhancing physical and mental health, preserving independence, and elevating overall quality of life across the lifespan. Seals *et al*[6] proposed the concept of optimal longevity, incorporating a compressed disease period at life's end. This innovative perspective has led to the emergence of geroscience, a new field in aging research. Geroscience is dedicated to identifying and intervening in biological mechanisms to enhance health span and actively promote healthy aging in individuals.

Aging itself is initiated by a combination of genetic and environmental factors that can influence organisms from birth. The signs of aging encompass various aspects, including impaired vision, hearing loss, muscle strength decline, reduced bone density, weakened immune system, cognitive decline, less efficient metabolism, reduced energy, hair loss, diminished balance, and overall decreased mobility[7].

Understanding these aging indicators is crucial within the setting of geroscience in order to promote extended health span and well-being throughout the aging process. In this review, we analyze the multifaceted roles of stem cells in tissue maintenance, disease pathogenesis, and the regulation of aging by comprehensively examining their properties, functions, and regulatory mechanisms *in vivo*.

## AGING AND IMPAIRED VISION

Older adults commonly face three visual problems: Impaired spatial contrast sensitivity, scotopic sensitivity loss, and delayed rod-mediated dark adaptation, along with reduced visual processing speed. While the extent of deficits varies among individuals, older adults are likely to experience one or more of these disturbances. These visual challenges

impact daily tasks. In severe cases, these aging-related visual issues may indicate the emergence of visual pathway conditions and diseases common in the elderly[8].

## AGING AND IMPAIRED HEARING

Age-related hearing loss (ARHL), or presbycusis, is a common occurrence in mammals, including humans, with varying onset times and degrees of loss. It manifests through reduced sensitivity to sound, especially high pitches, and a diminished ability to understand speech in background noise[9].

ARHL involves both peripheral structures of the inner ear and central acoustic pathways, with oxidative stress identified as a key pro-aging mechanism in the human cochlea[10].

## LOSS OF BONE DENSITY AND MUSCLE STRENGTH

Muscle and bone aging contribute significantly to morbidity and mortality in older populations, affecting the overall quality of life. Skeletal aging begins after reaching peak bone mass, with varying onset ages and rates between sexes. Two primary factors contribute to the decline in muscle mass and function with age: Muscle fiber atrophy and muscle fiber loss. It is evident that both these components exert influence on the regulation of muscle atrophy and dysfunction, impacting either individual muscles or groups of muscles[11].

Bone loss is accelerated during the perimenopausal period in women and gradually progresses in men of advanced age. Changes in both bone quantity and quality occur throughout growth and aging, impacting microarchitecture, size, and geometry. Genetic and epigenetic factors may predispose individuals to osteoporosis, characterized by weakened bones and an increased risk of fractures[12].

## WEAKENED IMMUNE SYSTEM

The concept of immune senescence reflects age-related changes in immune responses, both cellular and serological, affecting the process of generating specific responses to foreign and self-antigens. The decline of the immune system with age is reflected in the increased susceptibility to infectious diseases, weaker responses to vaccination, increased prevalence of cancer, autoimmune and other chronic diseases[13].

## AGING AT THE CELLULAR LEVEL

In 2013, López-Otín *et al*[14] defined nine cellular and molecular hallmarks of aging, laying a crucial foundation for future research in the field. These hallmarks encompass genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient-sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication (Figure 1). Expanding on this framework, a research symposium entitled "New Hallmarks of Aging" convened in Copenhagen on March 22, 2022. This symposium concentrated on presenting innovative findings and recontextualizing the original nine hallmarks, introducing potential new hallmarks such as compromised autophagy, dysregulation of RNA processing, microbiome disturbances, altered mechanical properties, and inflammation[14,15].

## STEM CELLS AND MESENCHYMAL STEM CELLS IN AGING

Stem cells, characterized by their immature nature, possess the remarkable ability to undergo infinite self-renewal and differentiate into various cell lineages. Their unique capacity to function as a reservoir for the production, maintenance, repair, and regeneration of diverse tissues distinguishes them from other cell types[16].

There are two main subtypes of stem cells: Embryonic stem cells, derived from early-stage embryos, and mesenchymal stem cells (MSCs), found in adults and isolated from various tissues such as bone marrow and adipose tissue.

As a subtype of adult stem cells, MSCs are multipotent stem cells with remarkable regenerative potential found in various tissues throughout the body. They play a pivotal role in tissue regeneration and can differentiate into multiple cell lineages. Amongst the myriad roles played by MSCs, one of the most captivating and promising aspects lies in their remarkable ability to counteract the effects of aging. MSCs can go beyond their traditional roles in tissue repair, immune modulation, and paracrine signaling to intricately engage in fighting the multifaceted processes associated with aging, making them particularly promising for future clinical trials, especially in the fields of regenerative medicine and anti-aging treatments[17-19].

In fact, MSCs are known for their important proliferation, especially if the donor is young since proliferation capacity declines with age. Also, they can be differentiated into osteoblasts, chondrocytes, myoblasts, adipocytes, and fibroblasts. Additionally, MSCs exhibited anti-aging benefits through the secretion of cytokines and growth factors that act as

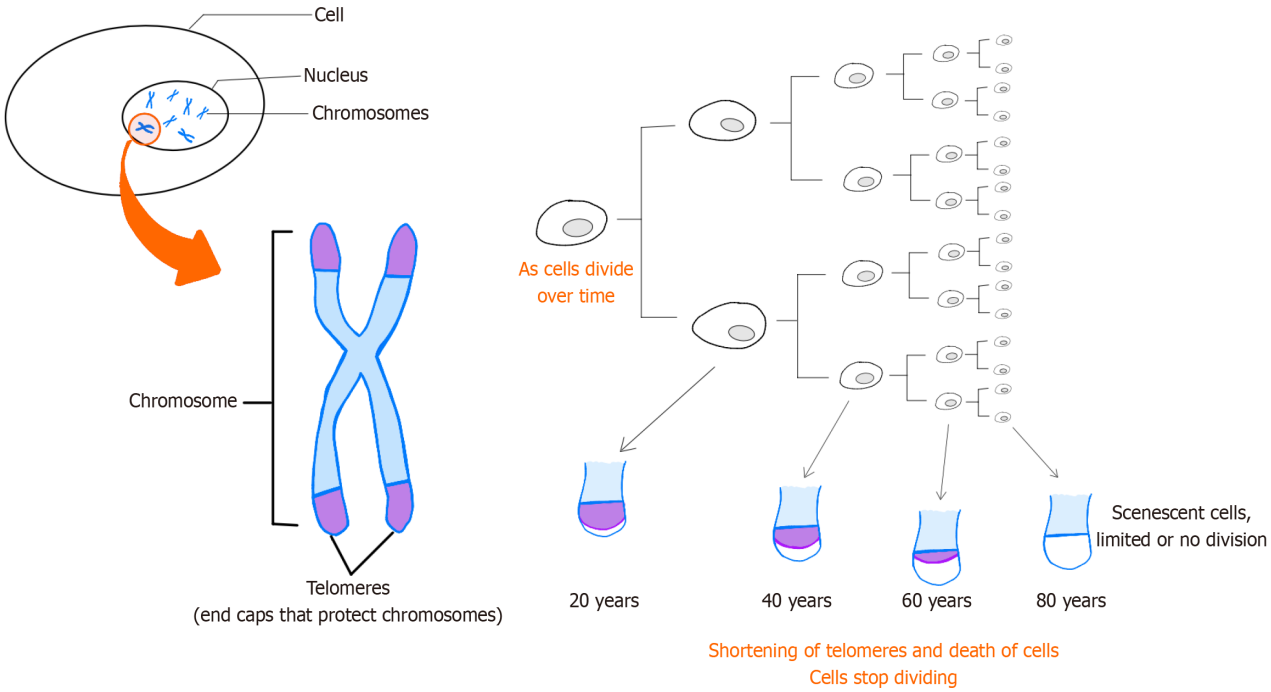


**Figure 1 Telomeres on the end of chromosomes are believed to protect the DNA strands and prevent them from fusing with other strands.** Telomeres lose a little of their length during each cell division. Since replicative DNA polymerases are not able to replicate telomeres, and telomerase is not expressed in normal mammalian somatic cells, telomeres become too short to replicate after a fixed number of cell divisions. Eventually, the cell will stop growing and enter cellular senescence.

promoters of angiogenesis, anti-inflammatory agents, and inhibitors of apoptosis[20,21].

In a recent study, Wang *et al*[22] highlighted the anti-aging and anti-obesity effects of MSCs from healthy mice when injected into older mice, providing new insights into potential anti-aging treatments. Moreover, MSCs, especially adipose stem cells, have been reported to improve aged skin by increasing angiogenesis growth factors[23].

MSCs reportedly release a wide array of bioactive molecules that collectively form the secretome. Within this complex mixture, an important subset is the exosomes which are tiny vesicles enriched with molecular cargo. These extracellular components, derived from the endosomal pathway within MSCs, constitute a vital facet of the broader secretome. The secretome, encompassing soluble factors such as growth factors and cytokines alongside exosomes, acts as a comprehensive communication system. Through paracrine signaling, MSCs influence neighboring and distant cells, fostering an environment conducive to tissue repair, immunomodulation, and anti-inflammatory responses. The interplay between MSCs, secretome, and exosomes highlights the interconnected web of regenerative mechanisms, underscoring their potential in therapeutic applications, from promoting wound healing to addressing aging-related degeneration[24-28].

These anti-aging effects encompass a spectrum of activities, from mitigating chronic inflammation to enhancing tissue regeneration and modulating cellular senescence[29]. The unique capacity of MSCs to address the underlying mechanisms of aging positions them at the forefront of regenerative medicine, holding immense potential for interventions, aiming to promote not just longevity, but a healthier and more vibrant aging process[30,31].

## TISSUE REGENERATION AND STEM CELL DIFFERENTIATION

MSCs are a subset of non-hematopoietic adult stem cells capable of differentiating into various cell types, including osteoblasts, chondrocytes, and adipocytes. This differentiation capacity and immunomodulatory properties contribute to tissue regeneration by replacing damaged or aging cells with new, functional cells. The differentiation potential of MSCs makes them promising candidates for regenerative medicine applications in repairing fragile tissues associated with the musculoskeletal system, nervous system, myocardium, liver, cornea, trachea, and skin[32-34].

## SECRETOME PRODUCTION AND IMMUNOMODULATION

As chronic inflammation is a hallmark of aging, MSCs exhibit immunomodulatory properties, suppressing excessive inflammatory responses by release of the secretome which contains a variety of anti-inflammatory cytokines and growth factors. These factors stimulate cell proliferation, enhance tissue repair, and contribute to overall health by modulating the immune system and creating an anti-inflammatory environment[35-37].

### *Intercellular communication*

Exosomes, derived from MSCs, contain bioactive molecules, including proteins, lipids, and nucleic acids. They act as messengers, transferring information between cells which can influence neighboring cells, promoting tissue repair and rejuvenation[38,39].

### *Anti-fibrotic effects*

MSCs and their secretome may have anti-fibrotic effects, reducing the accumulation of fibrotic tissue in organs. Fibrosis is associated with aging leading to impaired organ function. Hence, the therapeutic potential of MSCs resides in their capability to target multiple fibrogenesis parameters. This includes their capacity for immunosuppression, inhibition of the TGF-β1 pathway and mitigation of hypoxia and oxidative stress[40].

### *Cellular protection*

MSC-derived exosomes may carry antioxidant enzymes and other protective factors. This cargo can help protect cells from oxidative stress, which is associated with cellular damage and an accelerated aging process[41].

### *Mitochondrial function*

MSCs and exosomes may influence mitochondrial function, by enhancing energy production within cells. Indeed, improved mitochondrial function is associated with increased cellular resilience and longevity[42,43].

### *Senescence modulation*

MSCs and exosomes may modulate cellular senescence, the process by which cells lose their ability to divide and function properly. By influencing senescence, MSCs contribute to maintaining youthful cellular functions[36,44].

### *Extracellular matrix remodeling*

MSCs and secretome contribute to remodeling of the extracellular matrix, ensuring the maintenance of tissue structure, elasticity, and functionality[45].

### *Stem cell niche maintenance*

MSCs and secretome play a role in maintaining stem cell niches in tissues. This ensures a continuous pool of functional cells for tissue repair and regeneration[46].

### *Enhanced wound healing*

MSCs and exosomes contribute to enhanced wound healing, which is essential for preventing age-related complications and maintaining the integrity of the skin and other tissues in the elderly[38,46].

### *Angiogenesis promotion*

Growth factors found in the secretome can stimulate angiogenesis and the formation of new blood vessels. Improved blood flow is crucial for supplying nutrients to tissues and therefore supporting overall healthy tissues[47,48].

### *Promotion of autophagy*

Exosomes may stimulate autophagy, a cellular process that removes damaged components. Enhanced autophagy can contribute to cellular rejuvenation, thereby underlining their therapeutic efficacy. Consequently, strategies aimed at modulating autophagy through MSC-based therapies hold significant promise for enhancing therapeutic outcomes in various human diseases, including cancer, autoimmune disorders, and neurodegenerative conditions[49,50].

## CONCLUSION

As we delve into the intricacies of MSCs and their multifaceted roles, it becomes evident that these cells hold immense promise in revolutionizing the landscape of regenerative medicine especially in the elderly. MSCs have important characteristics that make them ideal candidates for use in regenerative medicine, such as immunomodulatory capability valuable for improving immune system abnormalities, paracrine or autocrine roles that produce growth factors, and the vital potential to differentiate into various cells. Also, there is a lack of anti-aging treatment derived from the mechanism of aging. This review showed that MSCs have a significant effect on delaying aging. This exploration of their roles provides a foundation for understanding their potential applications in addressing a wide array of health-related diseases and challenges. It is essential to note that research in this field is ongoing, and clinical applications are still being explored. Further studies are needed to understand the optimal conditions for MSC-based therapies and their long-term effects on aging-related conditions.

## ACKNOWLEDGEMENTS

We thank all who contributed to this work from all institutions.

## FOOTNOTES

**Author contributions:** Khalil C was responsible for conceptualization and supervision; Ghosn M, Khalil C, Chebly A and Bou Atme N were responsible for methodology; Khalil C and Rafoul P were responsible for validation; El Assaad N and Khalil Cwere responsible for formal analysis, data curation; Khalil C and Akouch K were responsible for writing-original draft preparation; Akouch K, Achkar R, Bou Atme N and Khalil Cwere responsible for visualization; El Assaad N, Hanna C, Salame R, Ghosn M, Abou Zeid S, Achkar R, Raffoul P, Hanna C, Chebly A and Khalil C were responsible for writing - review & editing; all authors have read and agreed to the submitted version of the manuscript.

**Conflict-of-interest statement:** The authors declare that they have no conflict of interest.



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**S-Editor:** Lin C
**L-Editor:** Webster JR
**P-Editor:** Wang WB

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*World J Exp Med* 2024 September 20; 14(3): 93742

DOI: 10.5493/wjem.v14.i3.93742

ISSN 2220-315x (online)

ORIGINAL ARTICLE

Retrospective Study

# Association of cannabis use disorder with atrial fibrillation in young men without concomitant tobacco use: Insights from nationwide propensity matched analysis

Bhavin Patel, Sumanth Khadke, Kshitij Mahajan, Avleen Dhingra, Rishika Trivedi, Samrath Singh Brar, Sakshi Dixit, Vaibhav Periwal, Shaylika Chauhan, Rupak Desai

**Specialty type:** Cardiac and cardiovascular systems

**Provenance and peer review:** Invited article; Externally peer reviewed.

**Peer-review model:** Single blind

**Peer-review report's classification**
**Scientific Quality:** Grade B
**Novelty:** Grade B
**Creativity or Innovation:** Grade B
**Scientific Significance:** Grade B

**P-Reviewer:** Mehta V

**Received:** March 8, 2024
**Revised:** June 23, 2024
**Accepted:** July 4, 2024
**Published online:** September 20, 2024
**Processing time:** 173 Days and 22.4 Hours

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## Abstract

*BACKGROUND*

Recent data are inconclusive regarding the risk of arrhythmias among young cannabis users. Furthermore, many young adults use both cannabis and tobacco, which could add a residual confounding effect on outcomes. So, we studied young men who have cannabis use disorder (CUD) excluding tobacco use disorder (TUD) to understand their independent association with atrial fibrillation (AF) and related outcomes.

***AIM***
To study the association of CUD with AF and related outcomes.

***METHODS***
We used weighted discharge records from National Inpatient Sample (2019) to assess the baseline characteristics and mortality rates for AF-related hospitalizations in young (18-44 years) men in 1:1 propensity-matched CUD + *vs* CUD- cohorts without TUD.

***RESULTS***
Propensity matched CUD + and CUD- cohorts consisted of 108495 young men in each arm. Our analysis showed an increased incidence of AF in black population with CUD. In addition, the CUD + cohort had lower rates of hyperlipidemia (6.4% *vs* 6.9%), hypertension (5.3% *vs* 6.3%), obesity (9.1% *vs* 10.9%), alcohol abuse (15.5% *vs* 16.9%), but had higher rates of anxiety (24.3% *vs* 18.4%) and chronic obstructive pulmonary disease (COPD) (9.8% *vs* 9.4%) compared to CUD-cohort. After adjustment with covariates including other substance abuse, a non-significant association was found between CUD + cohort and AF related hospitalizations (odd ratio: 1.27, 95% confidence interval: 0.91-1.78, $P = 0.15$).

***CONCLUSION***
Among hospitalized young men, the CUD + cohort had a higher prevalence of anxiety and COPD, and slightly higher proportion of black patients. Although there were higher odds of AF hospitalizations in CUD + cohort without TUD, the association was statistically non-significant. The subgroup analysis showed higher rates of AF in black patients. Large-scale prospective studies are required to evaluate long-term effects of CUD on AF risk and prognosis without TUD and concomitant substance abuse.

**Key Words:** Cannabis; Marijuana; Atrial fibrillation; Tobacco use disorder; Original research; Smoking



**Core Tip:** Among young hospitalized men, the cohort with Cannabis use disorder had a higher prevalence of anxiety and chronic obstructive pulmonary disease. Earlier studies have shown higher arrhythmia burden with cannabis use; however, this study population without concomitant tobacco use disorder was not associated with higher risk of atrial fibrillation when other sociodemographic and comorbid confounding variables were controlled. On subgroup analysis, blacks showed higher atrial fibrillation rates in the cannabis use disorder + arm.

**Citation:** Patel B, Khadke S, Mahajan K, Dhingra A, Trivedi R, Brar SS, Dixit S, Periwal V, Chauhan S, Desai R. Association of cannabis use disorder with atrial fibrillation in young men without concomitant tobacco use: Insights from nationwide propensity matched analysis. *World J Exp Med* 2024; 14(3): 93742
**URL:** https://www.wjgnet.com/2220-315x/full/v14/i3/93742.htm
**DOI:** https://dx.doi.org/10.5493/wjem.v14.i3.93742

## INTRODUCTION

Atrial fibrillation (AF) is the most frequently encountered cardiac arrhythmia in clinical practice. Current literature suggests that AF will affect 6-12 million people in the United States by 2050 and 17.9 million people in Europe by 2060[1]. In addition, AF is associated with significant morbidity and mortality, with a significant cost of health care burden[2]. On the other hand, the association of AF with substance use disorder has shown a significant higher burden in the young population with higher substance use, including cannabis use disorder (CUD)[3]. On a worldwide scale, cannabis is one of the most highly used recreational substances, which has detrimental effects on cardiovascular health, including an established association with various types of arrhythmias, acute myocardial infarction, and stroke[4-7]. Often, CUD is associated with other substance use, and it is difficult to solely find the effect of CUD on incidence or association with AF. Considering the increasing burden of AF in society, it is mandatory to search for preventable causes of AF, which is why we sought to find an association between CUD and AF hospitalizations in young men without concomitant tobacco use disorder (TUD), which might work as a confounder if we consider its association with AF in the literature[8].

## MATERIALS AND METHODS

### *Study data, design and population*

The study utilized data from the National Inpatient Sample (2019) to identify hospital admissions of young men (age 18-44 years) in the United States with AF. The NIS dataset, which is a component of the Healthcare Cost and Utilization Project includes 35 million in-hospital encounters that occur annually across more than 1000 nonfederal acute care hospitals in 48 states[9]. Since patient identifiers were not included, Institutional Review Board approval was not necessary for this study. We identified hospitalizations of young men (age 18-44 years), with and without CUD after excluding the records with known TUD and then divided the main cohort into CUD + and CUD- arms using International Classification of Diseases, tenth revision, clinical modification (ICD-10-CM). Using the codes (F17.2) and (O99.33), tobacco smoking status was determined. Cardiovascular and extracardiac comorbidities were identified using Elixhauser Comorbidity Indices and predefined comorbidity criteria in the existing database based on ICD-10 codes. The identification of CUD + was accomplished by utilizing the diagnostic codes F12.1x and F12.2x from the ICD-10-CM. It is important to note that the code F12.21, which pertains to dependence in remission, was excluded from the cohort. Since the NIS contains deidentified data, IRB approval was not required.

### *Study variables and co-morbidities*

We studied baseline characteristics including mean age, race, median household income and various comorbidities. We compared both CUD + and CUD- groups with different comorbidities to evaluate its effect on AF related hospitalizations.

### *Study outcomes*

Our primary outcome was the frequency and odds of AF related hospitalizations in young men without concomitant TUD with and without CUD by multivariable logistic regression analysis adjusted for sociodemographic and comorbid confounding variables. We also evaluated AF admission rates stratified by race, median household income (lowermost income quartile *vs* highest income quartile) and hospital region (Northeast, Midwest, South, and West).

### *Statistical analysis*

For analyzing and identifying any categorical associations, we employed the Pearson $\chi^2$ test, and the Mann-Whitney *U* test was utilized for continuous variables that had a non-normal distribution. To ensure significance, we set a *P* value threshold of 0.05. In accordance with the privacy guidelines of the Healthcare Cost and Utilization Project (HCUP), any cell sizes below 11 were not disclosed to uphold patient confidentiality. We performed a 1:1 propensity score-matched analysis focusing on baseline characteristics and relevant comorbidities (age at admission, race, median household income quartile, hospital location/teaching status, region, alcohol abuse and other concomitant substance abuse) employing the near-neighbor method and 0.01 caliper width. Multivariable logistic regression was performed to evaluate the odds of outcomes, adjusting for potential patient and hospital-level covariates and preexisting comorbidities relevant to the studied patient population. We utilized the weighted data and complex survey modules in IBM SPSS Statistics v25.0 to accurately account for the sampling design and generate national estimates.

## RESULTS

Our initial analysis included 1388130 young men hospitalization with AF in total without TUD with and without CUD. Before matching, CUD + cohort experienced lower rate of AF-related hospitalizations compared to CUD- cohort 0.3% (395/1202400) *vs* 0.6% (7305/1267890); $P < 0.001$.

We performed 1:1 propensity-score matched analysis to obtain CUD + arm and CUD- arm with 108495 patients in each arm. post-matching the CUD + arm demonstrated higher rates of AF admissions *vs* CUD- arm (0.4% *vs* 0.3%, $P = 0.023$). On subgroup analysis, whites and Hispanics had comparable AF admission rates between CUD + *vs* CUD- arms, whereas blacks showed higher AF rates in the CUD + arm (0.3% *vs* 0.1%, $P = 0.001$). On income-based stratification, individuals from lowermost income quartiles have a slightly higher AF admission rate for CUD + arm compared to CUD- arm (0.4% *vs* 0.3%, $P = 0.023$). Conversely, individuals from the highest income quartile exhibited a slightly lower admission rate for CUD + arm compared to CUD- arm (0.2% *vs* 0.3%, $P = 0.020$). Southern region hospitals showed a higher rate of AF admission among CUD + cohort compared to CUD- cohort (0.4% *vs* 0.3%, $P = 0.03$) whereas admissions in young men in hospitals from the other region demonstrated comparable rates of AF ($P > 0.05$).

As shown in Table 1, Both CUD + and CUD- cohorts among young men without TUD had median age of 30 (24-37). It was evident that the CUD + more often had black patients in comparison to CUD- cohort (25.70% *vs* 25.50%). There was no significant difference in median household income in both arms. In both the arms highest number of patients were covered by Medicaid (40.40% *vs* 40.40%) and self-pay was higher in CUD + arm (15.70% *vs* 13.60%, $P < 0.0001$). CUD + cohort had higher association with chronic pulmonary disease (9.80% *vs* 9.40%, $P = 0.01$) and depression (12.20% *vs* 11.80%, $P = 0.007$) in compared to CUD- cohort. Surprisingly, CUD + patients had significantly low associated comorbidities including complicated hypertension (5.30% *vs* 6.30%), hyperlipidemia (6.40% *vs* 6.90%), obesity (9.10% *vs* 10.90%), peripheral vascular disease (0.90% *vs* 1.40%), alcohol abuse (15.50% *vs* 16.90%), diabetes with complications (6.40% *vs* 6.80%), prior myocardial infarction (1.00% *vs* 1.10%), prior stroke (1.10% *vs* 1.40%), prior venous thromboembolism (1.80% *vs* 3.20%), and cancer (1.60% *vs* 2.80%) when compared to CUD- patients.

**Table 1 Baseline characteristics of young men hospitalizations without concomitant tobacco use disorder with *vs* without cannabis use disorder, 2019, *n* (%)**

| Variable | | Cannabis use disorder | | *P* value |
|---|---|---|---|---|
| | | No (*n* = 108495) | Yes (*n* = 108495) | |
| Age (years) at admission | Median (IQR) | 30 | 30 | < 0.001 |
| Race | | | | < 0.001 |
| | White | 53525 (49.30) | 53365 (49.20) | |
| | Black | 27670 (25.50) | 27830 (25.70) | |
| | Hispanic | 19480 (18.00) | 19465 (17.90) | |
| | Asian or Pacific Islander | 1605 (1.50) | 1840 (1.70) | |
| | Native American | 1200 (1.10) | 1190 (1.10) | |
| Median household income national quartile for patient ZIP code | | | | 0.100 |
| | 0-25$^{th}$ | 38785 (35.70) | 38400 (35.40) | |
| | 26-50$^{th}$ | 26595 (24.50) | 26730 (24.60) | |
| | 51-75$^{th}$ | 24615 (22.70) | 24500 (22.60) | |
| | 76-100$^{th}$ | 18500 (17.10) | 18865 (17.40) | |
| Primary payer | | | | < 0.001 |
| | Medicare | 10325 (9.50) | 9365 (8.70) | |
| | Medicaid | 43715 (40.40) | 43730 (40.40) | |
| | Private insurance | 32395 (29.90) | 31465 (29.10) | |
| | Self-pay | 14675 (13.60) | 17045 (15.70) | |
| | No charges | 1385 (1.30) | 1455 (1.30) | |
| | Other | 5750 (5.30) | 5180 (4.80) | |
| Hypertension, complicated | | 6835 (6.30) | 5700 (5.30) | < 0.001 |
| Hypertension, uncomplicated | | 16735 (15.40) | 16335 (15.10) | 0.010 |
| Hyperlipidemia | | 7445 (6.90) | 6900 (6.40) | < 0.001 |
| Obesity | | 11780 (10.90) | 9920 (9.10) | < 0.001 |
| Peripheral vascular disease | | 1510 (1.40) | 970 (0.90) | < 0.001 |
| Chronic pulmonary disease | | 10235 (9.40) | 10590 (9.80) | 0.010 |
| Alcohol abuse | | 18325 (16.90) | 16770 (15.50) | < 0.001 |
| Drug abuse | | 55370 (51.00) | 55380 (51.00) | 0.960 |
| Diabetes with chronic complications | | 7375 (6.80) | 6890 (6.40) | < 0.001 |
| Diabetes without chronic complications | | 3735 (3.40) | 2950 (2.70) | < 0.001 |
| Prior myocardial infarction | | 1170 (1.10) | 1070 (1.00) | 0.030 |
| Prior transient ischemic attack/stroke | | 1570 (1.40) | 1165 (1.10) | < 0.001 |
| Depression | | 12800 (11.80) | 13210 (12.20) | 0.007 |
| Prior VTE | | 3475 (3.20) | 1910 (1.80) | < 0.001 |
| Cancer | | 3030 (2.80) | 1745 (1.60) | < 0.001 |

$P < 0.05$ indicates statistical significance. IQR: Interquartile range, VTE: Venous thromboembolism.

Unadjusted odds of AF in the CUD + cohort without concomitant TUD were 1.19 with a 95% confidence interval (95%CI) of 0.85 to 1.66 and failed to reach a statistical significance; $P$ = 0.313. Furthermore, when adjusted for confounders, the odds of AF hospitalizations in CUD + cohort without TUD were non-significant (Odd ratio: 1.27, 95%CI: 0.91-1.78, $P$ = 0.15) (Table 2).

## DISCUSSION

This study observed a relative increase in AF-related hospitalizations, anxiety, and chronic obstructive pulmonary disease (COPD) rates among the propensity matched CUD + subgroup compared to CUD- group even in absence of concomitant TUD in young, hospitalized men. After adjusting for demographic factors and comorbidities, the odds of AF-related hospitalization were 27% higher in the CUD + cohort compared to the CUD-cohort, though this difference was not statistically significant.

The mechanisms linking CUD to AF may involve direct toxic effects on the myocardium and autonomic nervous system, causing arrhythmogenic effects. Theoretically, THC stimulation increases the content of catecholamines and adrenaline in cardiac tissue, which may promote arrhythmias. Moreover, it is evident that cannabis increased action potential and decreased the rapid delayed rectifier potassium currents, the slow delayed rectifier potassium currents, and the transient outward rectifier potassium currents, which promote arrhythmias[10,11]. The CUD + group also had higher anxiety and stress, possibly contributing to arrhythmogenic triggers. Of note, the prevalence of co-diagnosed CUD and AF has risen substantially in recent years, which is concerning given the ongoing expansion of cannabis legalization[12]. In addition, a recent scientific statement from the American Heart Association suggests potential cardiovascular side effects of CUD by stimulating CB1R, causing sympathetic activation, myocyte hypertrophy, reduced mobility, and chronic endothelial dysfunction, leading to direct effects such as cardiomyopathy, acute myocardial infarction, and arrhythmias[13].

Importantly, this study highlights CUD as a potential contributor to racial and socio-economic disparities in AF outcomes[14]. The higher burden of CUD and related AF admissions in blacks warrants greater attention, as this could compound their underlying thromboembolic risk due to prothrombotic milieu, hyperactivation of adrenergic drive, and can potentiate conversion from paroxysmal to persistent AF[12]. Considering the younger age group of CUD + patients and the progression from paroxysmal to permanent AF with a low burden of hypertension, hyperlipidemia, diabetes, obesity, and alcohol use disorder, progressing from paroxysmal to persistent AF can escalate subsequent cardioembolic complications in higher-risk groups. Southern region hospitals reported a higher rate of AF admission among the CUD + cohort compared to the CUD-cohort, highlighting potential geographical factors contributing to variations in the cannabis-cardiovascular risk relationship. Regional differences could be due to existing differences in state-wise cannabis policies and decriminalization laws. Furthermore, it is important to consider the potential impact of different cannabis strains and potency levels, as these factors can vary across regions. Additionally, variations in healthcare access and quality between different regions may have influenced the rates of AF admission among individuals with CUD. Efforts to reduce the burden of CUD and AF among Black individuals should focus on addressing the root causes of these disparities, including socioeconomic disadvantage, limited access to healthcare, and exposure to environmental stressors [8]. Interventions that promote health equity, such as community-based education programs and policies that reduce barriers to healthcare access, may be particularly effective in mitigating the adverse cardiovascular effects of cannabis use in vulnerable populations[15].

The co-diagnosis of CUD and AF has risen substantially from 2008 to 2018, with over a 3-fold relative increase and a nearly 5-fold absolute increase[12]. However, previous studies have not considered the confounding effect of TUD, which has profound cardiac adverse effects[12]. Moreover, smoking is associated with a 2-fold increased risk of AF attributed to current smoking and a trend towards a lower incidence of AF amongst smokers who quit[16]. With the legalization of cannabis across many states, this trend is expected to increase and have a growing impact on inpatient healthcare utilization and costs[12]. However, the cost of care for these CUD patients with AF was substantially higher in this study, suggesting a pathologic interaction that results in more severe AF requiring intensive treatment. As these younger patients with CUD and AF age, they may develop risk factors like hypertension and coronary disease that promote AF progression and complications like stroke.

Further research is needed to characterize CUD patterns, mechanisms, and outcomes in AF patients. This includes a detailed assessment of cannabis use history, arrhythmia phenotype, longitudinal outcomes, and potential treatment implications such as cannabis cessation. A focus on evaluating women, Hispanics, and other minority populations will also expand the generalizability. Future research should aim to explore these regional differences in more detail in order to gain a comprehensive understanding of the complex relationship between cannabis use, cardiovascular health, and geographic factors. Further study of the CUD-AF relationship offers significant potential to understand and reduce population health burdens related to cannabis use and AF.

Additional research using longitudinal designs is needed to clarify the temporality and quantify the magnitude of the association between cannabis use and AF risk. Understanding these relationships has important public health implications, given rising rates of cannabis use and growing awareness of AF-related morbidity in young people. Future studies should employ geospatial analysis techniques to map the distribution of CUD and AF across different regions and to identify hotspots where targeted interventions may be most effective[17]. Collaborations between researchers, policymakers, and healthcare providers will be essential in developing region-specific strategies to address the CUD-AF relationship and to promote cardiovascular health in communities most affected by cannabis use[18].

**Table 2 Odds of atrial fibrillation related admissions in young men without concomitant tobacco use disorder with *vs* without cannabis use disorder by logistic regression**

| Item | OR | 95%CI lower limit | 95%CI upper limit | *P* value |
|---|---|---|---|---|
| Unadjusted | 1.19 | 0.85 | 1.66 | 0.313 |
| Adjusted for age, race, income quartile, hospital location/teaching status, and regions | 1.19 | 0.85 | 1.67 | 0.300 |
| Adjusted for all baseline patient level and hospital level sociodemographic characteristics and pre-existing comorbidities | 1.27 | 0.91 | 1.78 | 0.158 |

$P < 0.05$ indicates statistical significance. Multivariable regression models were adjusted for age at admission, race, median household income national quartile for patient ZIP Code, location/teaching status of the hospital, region of the hospital, alcohol abuse, other drug abuse, depression, complicated hypertension, diabetes with chronic complications, hyperlipidemia, obesity, peripheral vascular disease, prior myocardial infarction, chronic pulmonary disease, prior venous thromboembolism, and cancer. 95%CI: 95% confidence interval; OR: Odds ratio.

In addition to AF, CUD is also associated with increased risk of anxiety and other mental health condition. Recent evidence presented at the 2023 Cannabis Clinical Outcomes Research Conference highlights the complex relationship between cannabis use and mental health outcomes. While some studies suggest potential therapeutic benefits of cannabis for certain mental health conditions, such as insomnia[18], others have found no significant improvement in pain, anxiety, or depression among adolescents and young adults[18]. These findings underscore the need for further research to elucidate the effects of cannabis on mental health and cognition across different populations and contexts.

Recent studies have also suggested a potential link between cannabis use and COPD exacerbations. In a study by Chatkin *et al*[19], patients with COPD who reported using cannabis had a higher risk of exacerbations compared to non-users. Similarly, Tan *et al*[20] found that heavy cannabis use was associated with an increased risk of COPD exacerbations in a population-based cohort. Our study found a relative incremental association between cannabis use and COPD exacerbation rates similar to the potential for increased risk reported in other studies[19,20].

This study has limitations, including reliance on administrative data lacking clinical granularity, diagnostic coding inconsistencies, and the inability to infer causation due to the cross-sectional design. Other unmeasured confounders may include healthcare access and substance use patterns. Nonetheless, these findings highlight CUD as an emerging risk factor for AF among young adults, particularly in minority populations at elevated risk of AF complications. Additional longitudinal research is warranted to elucidate the temporal associations and detailed mechanisms linking cannabis use and arrhythmogenesis across demographically diverse populations.

## CONCLUSION

This nationwide study highlights the effects of CUD on AF hospitalizations in young men without confounding effects of TUD. There was no association with higher AF risk when other confounding variables were controlled. Young black patients with CUD had higher rates of AF-related admissions. Large-scale prospective studies are required to evaluate long-term effects of CUD on AF risk and prognosis without TUD and concomitant substance abuse and to help formulate preventive measures to reduce AF burden in the young population.

## FOOTNOTES

**Author contributions:** Patel B contributed to writing-original draft, writing-review, editing, visualization, and project administration; Khadke S contributed to writing-original draft, writing-review, and editing; Mahajan K contributed to writing-original draft, writing-review, editing, and data curation; Dhingra A contributed to writing-original draft, writing-review, editing, data curation, and visualization; Trivedi R, Brar SS, Dixit S, and Periwal V contributed to writing-review, editing, data curation, and visualization; Chauhan S contributed to writing-review, editing, project administration, and supervision; Desai R contributed to conceptualization, methodology, software, formal analysis, resources, data curation, writing-original draft, writing-review, editing, project administration, and supervision.

**Institutional review board statement:** Data is obtained from a publicly available data set, so patient identifiers are not used.

**Informed consent statement:** Informed consent statement was not needed because the study was carried out on a publicly available data set without patient identifiers.

**Conflict-of-interest statement:** The authors have no conflict of interest related to the manuscript.

**Data sharing statement:** Technical appendix, statistical code, and dataset available from the corresponding author on request.

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**S-Editor:** Chen YL
**L-Editor:** A
**P-Editor:** Cai YX

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*World J Exp Med* 2024 September 20; 14(3): 94999

DOI: 10.5493/wjem.v14.i3.94999

ISSN 2220-315x (online)

ORIGINAL ARTICLE

Retrospective Study

# *SCN1A* rs6732655A/T polymorphism: Diagnostic and therapeutic insights for drug-resistant epilepsy

Aroop Viswas, Pradeep K Dabla, Dharmsheel Shrivastav, Swapan Gupta, Manisha Yadav, Subhash Yadav, Bidhan Chandra Koner

**Specialty type:** Medicine, research and experimental

**Provenance and peer review:** Invited article; Externally peer reviewed.

**Peer-review model:** Single blind

**Peer-review report's classification**
**Scientific Quality:** Grade C
**Novelty:** Grade B
**Creativity or Innovation:** Grade B
**Scientific Significance:** Grade B

**P-Reviewer:** Wei X

**Received:** March 29, 2024
**Revised:** May 20, 2024
**Accepted:** June 21, 2024
**Published online:** September 20, 2024
**Processing time:** 152 Days and 13.4 Hours

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## Abstract

***BACKGROUND***

A significant subset of individuals with epilepsy fails to respond to currently available antiepileptic drugs, resulting in heightened mortality rates, psychosocial challenges, and a diminished quality of life. Genetic factors, particularly within the *SCN1A* gene, and the pro-inflammatory cytokine response is important in intricating the drug resistance in idiopathic epilepsy cases. In this extended study, we determined the correlation of rs6732655A/T single nucleotide polymorphism to understand the causative association of *SCN1A* gene with epilepsy drug resistance and inflammatory response.

***AIM***

To find the correlation of *SCN1A* gene rs6732655A/T polymorphism with the drug-resistant epilepsy and inflammatory response.

***METHODS***

The study enrolled 100 age and sex-matched patients of both drug-resistant and

drug-responsive epilepsy cases. We analysed the rs6732655A/T polymorphism to study its association and causative role in drug-resistant epilepsy cases using restriction fragment length polymorphism technique. The diagnostic performance of interleukin (IL)-1β, IL-6, and high mobility group box 1 (HMGB1) protein levels was evaluated in conjunction with genotypic outcome receiver operating characteristic analysis.

***RESULTS***

AT and AA genotypes of rs6732655 *SCN1A* gene polymorphism were associated with higher risk of drug resistance epilepsy. Serum biomarkers IL-6, IL1β and HMGB1 demonstrated diagnostic potential, with cutoff values of 4.63 pg/mL, 59.52 pg/mL and 7.99 ng/mL, respectively, offering valuable tools for epilepsy management. Moreover, specific genotypes (AA and AT) were found to be linked to the elevated levels of IL-1β and IL-6 and potentially reflecting increased oxidative stress and neuro-inflammation in drug-resistant cases supporting the previous reported outcome of high inflammatory markers response in drug resistance epilepsy.

***CONCLUSION***

SCN1A genotypes AA and AT are linked to higher drug-resistant epilepsy risk. These findings underscore the potential influence of inflammation and genetics on epilepsy treatment resistance.

**Key Words:** Epilepsy; Drug resistance; *SCN1A* gene; Pro-inflammatory cytokines; Genetic factors



**Core Tip:** Genetic factors, including *SCN1A* gene variants, and their pro-inflammatory cytokine response [interleukin (IL)-1β, IL-6, and high mobility group box 1 protein (HMGB1)], play crucial roles in drug-resistant epilepsy. This study investigates the correlation between *SCN1A* gene variants (rs6732655A/T) and drug resistance in epilepsy, confirming higher levels of IL-1β, IL-6, and HMGB1 in drug-resistant cases and suggesting specific genotypes (AA and AT) as potential biomarkers for oxidative stress and neuro-inflammation in drug-resistant idiopathic epilepsy.

**Citation:** Viswas A, Dabla PK, Shrivastav D, Gupta S, Yadav M, Yadav S, Koner BC. *SCN1A* rs6732655A/T polymorphism: Diagnostic and therapeutic insights for drug-resistant epilepsy. *World J Exp Med* 2024; 14(3): 94999
**URL:** https://www.wjgnet.com/2220-315x/full/v14/i3/94999.htm
**DOI:** https://dx.doi.org/10.5493/wjem.v14.i3.94999

## INTRODUCTION

Epilepsy is a prevalent neurological disorder afflicting millions of people worldwide. Despite eminent progress in therapeutic approaches, it still remains an important challenge in the era of modern medicine. It is characterized by recurrent and unprovoked seizures, which occur due to abnormal electrical discharge within the brain. These seizures can have a profound impact on the lives of those affected, disrupting their daily activities and impairing their overall quality of life. While numerous antiepileptic drugs (AEDs) are available to manage these seizures, a significant subset of epilepsy patients continues to experience treatment resistance, representing a critical and unsolved problem in the field of epilepsy care[1]. Individuals with drug-resistant epilepsy endure not only the persistent burden of uncontrolled seizures but also face heightened mortality rates, psychosocial challenges, and a notable reduction in their overall well-being. Tragically, they are at a heightened risk of sudden unexpected death in epilepsy (commonly referred to as SUDEP), a devastating outcome that further emphasizes the urgency of comprehending and effectively addressing drug-resistant epilepsy[2]. The complexity of drug-resistant epilepsy arises from its multifaceted nature, involving intricate interplay of genetic, environmental, and pharmacological factors. Among these factors, genetic predispositions have drawn significant attention as potential contributors to the development of drug resistance. Understanding the genetic underpinnings of drug resistance is of paramount importance, as it may hold the key to unlocking novel therapeutic approaches and personalized treatment strategies tailored to individual patients[3].

Numerous single nucleotide polymorphisms (SNPs) have been investigated in the context of drug-resistant epilepsy. These studies have primarily focused on genes related to multidrug resistance, such as multi-drug resistance 1 (*MDR1*) and multidrug resistance-associated protein 2 (*MRP2*), voltage-gated sodium channel subunits, including *SCN1*, *SCN2*, and *SCN3*, and metabolic enzymes like *CYP2* and *CYP3*. The significance of voltage-gated sodium channels in this context is particularly noteworthy, given their relevance to AEDs that target sodium channels[4]. Recently, five novel *de novo* and inherited pathogenic/likely pathogenic *SCN1A* variants were identified in a Moroccan child with Dravet syndrome through exome analysis and confirmed by Sanger sequencing, including a novel pathogenic splice site variant (*SCN1A* c.965-2A>G). These findings enrich the mutations database for this major epilepsy gene and provide critical reference data to guide genetic counselling for *SCN1A*-related epilepsy disorders ranging from mild to severe phenotypes like Dravet syndrome[5,6]. Previous studies have investigated potential associations between genetic polymorphisms in the *SCN1A* gene and drug resistance in epilepsy, with some reporting significant findings. Margari *et al*[7] found that the AA

genotype of the *SCN1A* rs6732655A/T polymorphism was associated with an increased risk of drug-resistant epilepsy.

In our previous study, we found an association between rs10167228A/T SNP in the *SCN1A* gene and drug resistance in epilepsy. There are several genetic variants of interest due to their potential implications in the development of drug resistance in the context of idiopathic generalized epilepsy (IGE)[8].

In this study, we focused on the rs6732655A/T SNP in the *SCN1A* gene and its interactions that may lead to a pro-inflammatory response. Furthermore, we recently reported the causative association of rs10167228A/T SNP of *SCN1A* gene as a risk factor for drug-resistant epilepsy. We also found that high mobility group box 1 protein (HMGB1), interleukin (IL)-1, and IL-6 levels were significantly high in drug-resistant cases compared to drug-responsive patients[8].

In this study, we aimed to determine the correlation of specific genetic polymorphisms SNPrs6732655A/T within the *SCN1A* gene, which encodes a crucial sodium channel subunit and is intimately related to AEDs designed to act on these sodium channels. In addition to the genetic aspect, we also delve into the intricate web of neuro-inflammation, an emerging area of research in the epilepsy landscape. We investigated the serum concentrations of the proinflammatory cytokines IL-1β and IL-6, as well as HMGB1 and their interaction with *SCN1A* polymorphism genotypes. These markers are known to play pivotal roles in the inflammatory responses of the central nervous system and could offer valuable insights into the pathogenesis of drug-resistant epilepsy.

## MATERIALS AND METHODS

The study was performed out at the Department of Biochemistry and Department of Neurology, Govind Ballabh Pant Institute of postgraduate medical education and research, tertiary care super specialty hospital New Delhi, India. It involved a total of 100 age- and sex-matched cases of 50 drug-resistant epilepsy patients (group A) and 50 drug-responsive epilepsy patients (group B). These patients were enrolled from the inpatient department and outpatient department of the Department of Neurology with informed consent. The patients underwent diagnosis and classification according to the guidelines provided by the International League Against Epilepsy. Detailed histories of ethnicity, seizure frequency, duration of seizures, and compliance were recorded[6]. Patients with severe adverse drug reactions, poor compliance with AEDs, or an unreliable record of seizure frequency were excluded from the study. The study was conducted in compliance the Declaration of Helsinki of the World Medical Association (revised October 2013).

### *Sample collection and processing*

A total of 5 mL venous blood was collected under aseptic conditions. Blood samples were collected in plain vials and in ethylenediamine tetra acetic acid vials for biochemical parameters and inflammatory cytokine level analysis. The levels of serum IL-1β and IL-6 were estimated using a commercial enzyme-linked immunosorbent assay (ELISA) kit.

A total of 3 mL peripheral whole blood samples underwent DNA extraction *via* the column binding method utilizing the ReliaPrep™ Blood gDNA Miniprep System. A meticulous 1% agarose gel was prepared for electrophoresis, and the analysis of the rs6732655A/T polymorphism, the relevant region of the *SCN1A* gene was amplified *via* polymerase chain reaction (PCR) using specific primers (Table 1) and PCR conditions. PCR products were subjected to digestion with *Taq*I restriction endonuclease enzyme. This restriction fragment length polymorphism (RFLP)-PCR process exhibited specific outcomes for different alleles: The A allele remained uncut, yielding a single 250 bp band, while the T allele was cleaved into two smaller fragments (95 and 155 bp). Heterozygotes displayed a combination of both alleles, generating bands of 250 bp, 155 bp, and 95 bp. After PCR amplification, the products were resolved through electrophoresis in a 3% agarose gel (Figure 1).

### *Statistical analysis*

Data analysis was conducted using SPSS version 21 (IBM Corp., Armonk, NY, United States). Quantitative data were expressed using mean, standard deviation, frequency, and percentage for qualitative data. An independent *t*-test was used to compare two independent variables, and the normality of the data was assessed using the Kolmogorov-Smirnov test. Parametric and non-parametric variables were compared using Student *t*-tests, ANOVA, Fisher's exact test, Mann-Whitney *U* tests and Kruskal Wallis test. Receiver operating characteristic (ROC) curve analysis was employed for prediction analysis and used to measure the specificity and sensitivity. Statistical significance was set at $P < 0.05$ for all tests.

## RESULTS

In our previous report, we found that the mean age in the drug-resistant IGE group (cases) and the drug-responsive IGE group (controls) was 27.30 ± 7.0 years (26 males and 24 females) and 22.74 ± 7.3 years (21 males and 29 females), respectively. The serum levels of IL-1β were 91.81 (65.09-126.94) pg/mL *vs* 35.23 (15.05-43.87) pg/mL; IL-6 was 7.15 (5.0-18.13) pg/mL *vs* 2.97 (2.44-4.03) pg/mL; and HMGB-1 was 11.29 (9.11-16.93) ng/mL *vs* 6.02 (4.44-7.87) ng/mL, all of which were significantly higher in drug-resistant cases compared to drug-responsive cases, respectively[8].

### *Distribution of rs6732655 gene polymorphism*

Our investigation for the rs6732655 polymorphism revealed that within the drug-resistant group, nine subjects (18%) had the AA genotype, 17 subjects (34%) possessed the AT genotype, and 24 subjects (48%) carried the TT genotype. In

**Table 1 Primers for single nucleotide polymorphism analysis**

| SNP | Primers | Sequence | Restriction enzyme |
|---|---|---|---|
| rs6732655A/T | Forward primer | 5′-CCAAATGGTGACACAGTGAA-3′ | *Rsa*I |
| | Reverse primer | 5′-GCCTTGATCACTTGTAGGACTTTT-3′ | |

SNP: Single nucleotide polymorphism.

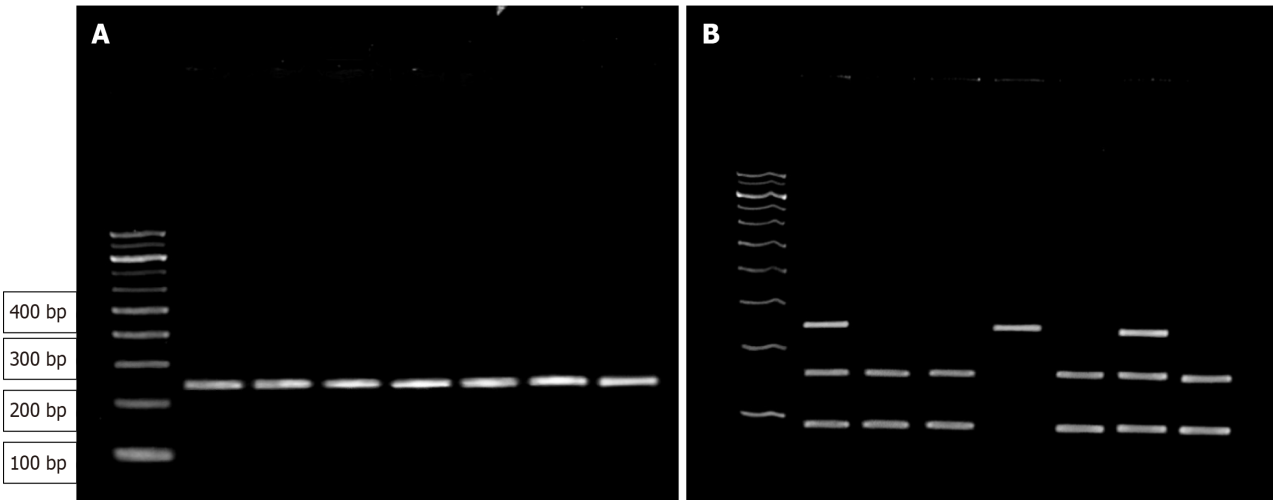


**Figure 1 Restriction fragment length polymorphism-polymerase chain reaction for rs6732655A/T polymorphism.** A: Amplified products electrophoresed on 2% agarose gel, 250 bp amplicons obtained; B: *Taq*I-digested products electrophoresed on 3% agarose gel [Lanes 1 to 8 from left to right: Lane 1: 100 bp DNA ladder; Lane 5: Homozygous wild genotype (250 bp); Lanes 2 and 7: Heterozygous mutant genotypes (250, 155 and 95 bp); Lanes 3, 4, 6 and 8: Homozygous mutant genotypes (155 and 95 bp)].

contrast, within the drug-responsive group, five subjects (10%) exhibited the AA genotype, 24 subjects (48%) had the AT genotype, and 21 subjects (42%) bore the TT genotype. Similarly, when comparing the distribution of rs6732655 genotypes across both study groups, our analysis demonstrated no statistically significant difference, with a *P* value of 0.281 (Table 2).

### *Risk of drug resistance associated, rs6732655 genotypes*

Risk analysis was performed to examine the relationship between distinct rs6732655 genotypes and drug resistance. The AT genotype exhibited a higher risk association with an odds ratio of 1.58 [95% confidence interval (CI): 0.456-5.444]; (*P* value = 0.47). Conversely, a lower risk association was observed with the AA genotype, characterized by an odds ratio of 0.62 (95%CI: 0.264-1.456, *P* = 0.27) (Table 3).

**Serum levels of IL-1β, IL-6, and HMGB1 in rs6732655 genotype:** Analysis of the serum levels of IL-1β, IL-6, and HMGB1 in both drug-resistant and drug-responsive cases was performed. In the drug-resistant group, the median serum IL-1β levels for the AA, AT, and TT genotypes were 150.05 pg/mL (IQR: 114.35-194 pg/mL), 89.35 pg/mL (IQR: 81.55-105.22 pg/mL), and 71.36 pg/mL (IQR: 25.80-126.67 pg/mL), respectively. The level of serum IL-1β among the different genotypes was significantly different (*P* = 0.003). Similarly, the median serum IL-1β levels for the AA, AT, and TT genotypes within the drug-responsive group were 56.71 pg/mL (IQR: 48.97-57.77 pg/mL), 33.21 pg/mL (IQR: 15.18-40.68 pg/mL), and 30.00 pg/mL (IQR: 14.34-42.25 pg/mL), respectively. Similar to the IL-1β serum levels in drug-resistant patients, the levels of IL-1β were also significantly different among the different genotypes of the drug responsive cases (*P* = 0.004).

In the drug-resistant group, the median IL-6 levels serum were significantly different between the three genotypes ($P \leq 0.01$). The IL-6 values for the AT, AA, and TT genotypes were 29.06 pg/mL (IQR: 14.73-51.75 pg/mL), 6.89 pg/mL (IQR: 5.06-11.47 pg/mL), and 5.61 pg/mL (IQR: 3.17-11.49 pg/mL), respectively. In the three genotypes of the drug-responsive group, the IL-6 levels for AA, AT, and TT were 5.02 pg/mL (IQR: 4.48-8.27 pg/mL), 2.95 pg/mL (IQR: 2.17-3.77 pg/mL), respectively.

Furthermore, within the drug-resistant group, the median serum HMGB1 levels for the AA, AT, and TT genotypes were 17.21 ng/mL (IQR: 15.56-26.23 ng/mL), 10.72 ng/mL (IQR: 9.85-11.92 ng/mL), and 9.39 ng/mL (IQR: 7.89-19.92 ng/mL) respectively, with significant differences observed (*P* = 0.005). However, there was no significant difference in the median HMGB1 serum levels of the AA, AT, and TT genotypes [AA-7.98 ng/mL (IQR: 7.14-8.68), AT-5.86 ng/mL (IQR: 4.11-6.91 ng/mL), TT-5.92 ng/mL (IQR: 3.94-7.87 ng/mL; *P* = 0.06] (Table 4).

**Table 2 Distribution of rs6732655 gene polymorphism**

| SNP | Allele type | Drug resistant, *n* = 50 | Drug responsive, *n* = 50 | $\chi^2$ | *P* value |
|---|---|---|---|---|---|
| rs6732655 | AA | 9 (18) | 5 (10) | 2.538 | 0.281 |
| | AT | 17 (34) | 24 (48) | | |
| | TT | 24 (48) | 21 (42) | | |

Data are *n* (%). SNP: Single nucleotide polymorphism.

**Table 3 Risk of drug resistance associated with rs6732655 genotypes**

| SNP | Genotype | OR | 95%CI | *P* value |
|---|---|---|---|---|
| rs6732655 | AA | 0.62 | 0.264-1.456 | 0.27 |
| | AT | 1.58 | 0.456-5.444 | 0.47 |
| | TT | Reference | Reference | Reference |

CI: Confidence interval; OR: Odds ratio; Ref: Reference polymorphism in OR analysis; SNP: Single nucleotide polymorphism.

**Table 4 Comparison of interleukin-1β, interleukin-6 and high mobility group box 1 protein in single nucleotide polymorphisms and alleles**

| Parameter | SNP | Allele type | Drug resistant, *n* = 50 | *P* value | Drug responsive, *n* = 50 | *P* value |
|---|---|---|---|---|---|---|
| IL-1β in pg/mL | rs6732655 | AA | 150.05 (114.35-194) | 0.003[a] | 56.71 (48.97-57.77) | 0.004[a] |
| | | AT | 89.35 (81.55-105.22) | | 33.21 (15.18-40.68) | |
| | | TT | 71.36 (25.80-126.67) | | 30.00 (14.34-42.25) | |
| IL-6 in pg/mL | rs6732655 | AA | 29.06 (14.73-51.75) | 0.001[a] | 5.02 (4.48-8.27) | 0.005[a] |
| | | AT | 6.89 (5.06-11.47) | | 2.95 (2.17-3.77) | |
| | | TT | 5.61 (3.17-11.49) | | 2.82 (2.49-3.85) | |
| HMGB-1 in ng/mL | rs6732655 | AA | 17.21 (15.56-26.23) | 0.005[a] | 7.98 (7.14-8.68) | 0.067 |
| | | AT | 10.72 (9.85-11.92) | | 5.86 (4.11-6.91) | |
| | | TT | 9.39 (7.89-19.92) | | 5.92 (3.94-7.87) | |

[a]$P < 0.05$. *P* calculated by Kruskal Wallis test.
HMGB-1: High mobility group box 1 protein; IL-1β: Interleukin-1β; IL-6: Interleukin-6; SNP: Single nucleotide polymorphism.

### *Diagnostic performance of serum IL-1β, IL-6, HMGB11 levels in both groups: ROC curve analysis*

ROC analysis was conducted to evaluate the diagnostic performance of serum IL-1β, IL-6, and HMGB1 levels in distinguishing between drug-resistant and drug-responsive groups. The cutoff value for serum IL-1β levels was determined to be 59.52 pg/mL. Additionally, ROC analysis was performed to assess the diagnostic performance of serum IL-6 levels, with a cutoff value of 4.63 pg/mL. Furthermore, serum HMGB1 levels were also subjected to ROC analysis, revealing a cutoff value of 7.99 ng/mL. These analyses aimed to provide insights into the ability of these serum biomarkers to discriminate between drug-resistant and drug-responsive groups, offering potential diagnostic utility for epilepsy management (Table 5).

## DISCUSSION

Despite the availability of diverse AEDs, over one-third of epilepsy patients remain resistant to treatment, experiencing elevated mortality, psychosocial challenges, and reduced quality of life, often succumbing to SUDEP. Identifying SNPs associated with drug-resistant epilepsy is complex due to its multifaceted nature. Prominent SNPs studied include those in multidrug resistance genes (*MDR1*, *MRP2*), voltage-gated sodium channel subunits (*SCN1*, *SCN2*, *SCN3*), and metabolic enzymes (*CYP2*, *CYP3*). Notably, given its relevance to AEDs that target sodium channels, investigating SNPs in the

**Table 5 Receiver operating characteristic analysis of interleukin-1β and interleukin-6**

| Factor | AUC | Asymptotic 95%CI | | Optimum cutoff | Sensitivity | Specificity | *P* value |
|---|---|---|---|---|---|---|---|
| | | Lower bound | Upper bound | | | | |
| IL-1β | 0.880 | 0.808 | 0.952 | 59.52 | 80.0% | 100% | < 0.001 |
| IL-6 | 0.869 | 0.795 | 0.943 | 4.63 | 86.0% | 86.0% | < 0.001 |

AUC: Area under curve; CI: Confidence interval; IL-1β: Interleukin-1β; IL-6: Interleukin-6.

*SCN1A* gene offers promise in elucidating drug-resistant epilepsy mechanisms. In this research investigation, we have undertaken an examination of the potential involvement of genetic polymorphisms within the sodium channel *SCN1A* gene, specifically rs6732655A/T, as well as the serum concentrations of proinflammatory cytokines IL-1β and IL-6, along with HMGB1, in the context of IGE and its association with drug resistance.

In our previously study, we observed elevated levels of inflammatory markers and oxidative stress in the drug-resistant cases, the comparison of the rs10167228 genotype between drug-resistant and drug-responsive groups did not reveal a significant association[8]. In this study, assessment of specific genotype associations with drug resistance compared to drug-sensitive individuals revealed that the drug-resistant population had 48% TT genotype, 34% AT genotype, and 18% AA genotype (Table 3). Furthermore, we found that the rs6732655 polymorphism is associated with a 1.5-fold higher risk in individuals with the AT genotype (Table 4). Similarly, various studies have shown a correlation between the severity of epileptic seizures and increased levels of cytokines in the blood plasma (HMGB-1, TLR4, IL-1β, IL-1R1, and TNF-α) of patients with drug-resistant epilepsy[9,10]. Increased levels of cytokines have also been observed in cerebrospinal fluid (IL-1β)[11]. Abe *et al*[12] found a link between the *SCN1A* gene's functional polymorphism rs3812718 (c.603-91G>A) and pharmacological response in terms of carbamazepine (CBZ) and phenytoin effective dosage. A population-specific link with CBZ and multiple drug resistant epilepsy has been described for the *SCN1A* IVS5-91GA intronic polymorphism of the *SCN1A* gene. Margari *et al*[7] suggested that intronic polymorphisms rs6730344, rs6732655, and rs10167228 within the *SCN1A* gene may represent potential factors that increase the risk of drug resistance. Additionally, the presence of the AA and AT genotypes in the rs1962842 intronic polymorphism has also been identified as a contributing risk factor in the drug-resistant patient group. As a result, these *SCN1A* gene polymorphisms could have a significant impact on how individuals with drug-resistant epilepsy respond to AEDs, which holds important implications for clinical management[7]. Namazi *et al*[13] explored the potential impact of *SCN1A* gene polymorphisms on the plasma concentrations of CBZ and its active metabolite carbamazepine 10, 11-epoxide (CBZE) in epileptic patients. However, the results did not reveal a significant association between *SCN1A* gene polymorphisms and CBZ and CBZE levels. This suggests that other factors may play a more significant role in inter-individual variations in response to CBZ.

Further, in our study, the ROC curve analysis revealed a statistically significant area under curve (AUC) value of 0.86 ( $P < 0.001$), indicating high diagnostic accuracy with a sensitivity and specificity of 86% at an optimal cutoff value of 4.63 pg/mL for serum IL-6. Similarly, for serum IL-1β, ROC analysis yielded an AUC of 0.880 ($P < 0.001$), with a sensitivity of 80% and specificity of 100% at an optimal cutoff value of 59.52 pg/mL. Therefore, 4.63 pg/mL and 59.52 pg/mL can serve as valuable cutoff values for discriminating drug-resistant cases from drug-responsive subjects based on serum IL-6 and IL-1β, respectively (Figure 2).

Similarly, several authors reported that in drug-resistant temporal lobe epileptic tissues, increased NF-κB expression and annexin V-positive neurons particularly correlated with IL-6 levels[14]. HMGB1 levels were shown to increase approximately 3–4 h after a drug-resistant epilepsy episode, highlighting the potential of HMGB1 as a promising marker [15]. Zhu *et al*[16] suggested that HMGB1 has a predictive value > 9.625 ng/mL for epilepsy prognosis in children.

Further, we observed the associations between different genotypes of the rs6732655 gene polymorphism. Significant links were observed between different genotypes of the rs6732655 gene polymorphism and serum levels of IL-1β, IL-6, and HMGB1. Those with AA and AT genotypes demonstrated substantially higher levels of IL-1β and IL-6, indicative of potentially elevated oxidative stress and neuroinflammation compared to individuals with TT genotypes. HMGB1 levels, however, exhibited variation in drug-sensitive groups only among AA genotypes. As reported in our recent study, individuals with AA and AT genotypes exhibited significantly higher IL-1β and IL-6 levels, possibly attributed to increased oxidative stress and neuroinflammation compared to those with TT genotypes.

Our study investigates genetic polymorphisms associated alterations in the *SCN1A* gene and associated raised serum inflammatory markers in drug-resistant epilepsy cases. We found the positive associations between *SCN1A* gene polymorphism and drug resistance, as well as the significant diagnostic accuracy for IL-6 and IL-1β levels in identifying drug resistance. These findings advance our understanding of epilepsy mechanisms and diagnostics.

## CONCLUSION

The study highlights a potential association between inflammation and drug resistance in IGE, particularly in epilepsy patients resistant to treatment. Notably, specific genetic variants, such as *SCN1A* rs6732655A/T genotypes (AT and AA), may predispose individuals to heightened drug resistance risk. These findings emphasize the need for further exploration into the intricate interplay between genetic factors and inflammatory processes in shaping drug response in IGE, offering

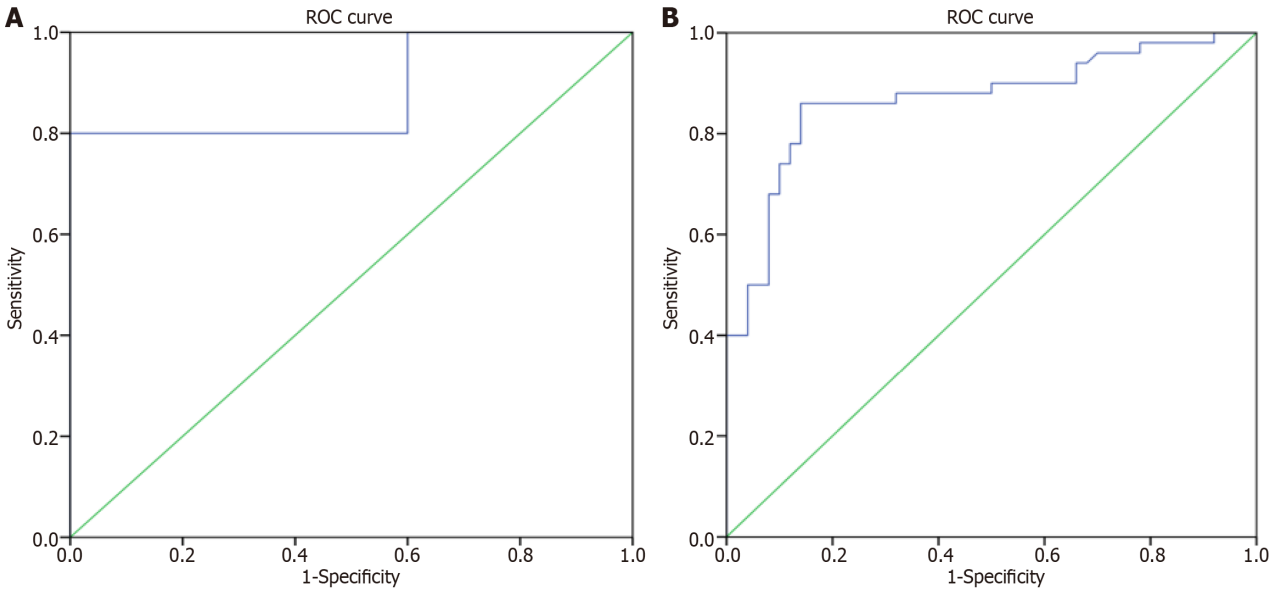


**Figure 2 Receiver operating characteristic analysis of serum interleukin-1β and interleukin-6 levels between drug resistant and drug responsive groups.** A: Interleukin-1β; B: Interleukin-6. ROC: Receiver operating characteristic.

promising avenues for advancing personalized treatment strategies and therapeutic interventions.

## ACKNOWLEDGEMENTS

Thanks to the Multidisciplinary Research Unit (MRU), Maulana Azad Medical College, New Delhi for providing molecular analysis research facilities and support.

## FOOTNOTES

**Author contributions:** Viswas A and Yadav S conducted experiments; Dabla PK designed the study and provided facilities for biochemical testing; Dabla PK, Shrivastav D, Viswas A, Yadav M analysed the manuscript; Koner BC provided facilities for molecular testing; Gupta S provided the facility for the enrolment of patients; All authors reviewed and approved the manuscript.

**Institutional review board statement:** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study involving human participants was approved by the Institutional Ethical Committee of Maulana Azad Medical College and associated hospitals, Delhi, India (F1/IEC/MAMC/82/10/2020/no.225; Dt-14.01.2021).

**Informed consent statement:** Informed consent was obtained from all individual participants included in the study. Personal interviews were conducted to gather information on ethnicity, seizure frequency, duration of seizures, and compliance.

**Conflict-of-interest statement:** All authors declared no conflicts of interest.

**Data sharing statement:** Technical appendix, statistical code, and dataset available upon reasonable request from the corresponding author at pradeep_dabla@yahoo.com.



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**S-Editor:** Fan M
**L-Editor:** Filipodia

**P-Editor:** Wang WB

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World Journal of Experimental Medicine

Submit a Manuscript: https://www.f6publishing.com

*World J Exp Med* 2024 September 20; 14(3): 95540

DOI: 10.5493/wjem.v14.i3.95540

ISSN 2220-315x (online)

ORIGINAL ARTICLE

Retrospective Study

# Platelet-to-neutrophil ratio predicts hemorrhagic transformation and unfavorable outcomes in acute ischemic stroke with intravenous thrombolysis

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**Specialty type:** Medicine, research and experimental

**Provenance and peer review:** Invited article; Externally peer reviewed.

**Peer-review model:** Single blind

**Peer-review report's classification**

**Scientific Quality:** Grade C

**Novelty:** Grade B

**Creativity or Innovation:** Grade C

**Scientific Significance:** Grade C

**P-Reviewer:** Arboix A, Spain

**Received:** April 12, 2024

**Revised:** May 22, 2024

**Accepted:** June 12, 2024

**Published online:** September 20, 2024

**Processing time:** 138 Days and 21.6 Hours

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## Abstract

### *BACKGROUND*

Acute ischemic stroke (AIS) retains a notable stance in global disease burden, with thrombolysis *via* recombinant tissue plasminogen activator (rtPA) serving as a viable management approach, albeit with variable outcomes and the potential for complications like hemorrhagic transformation (HT). The platelet-to-neutrophil ratio (P/NR) has been considered for its potential prognostic value in AIS, yet its capacity to predict outcomes following rtPA administration demands further exploration.

### *AIM*

To elucidate the prognostic utility of P/NR in predicting HT and clinical outcomes following intravenous rtPA administration in AIS patients.

### *METHODS*

Data from 418 AIS patients treated with intravenous rtPA at Thammasat University Hospital from January 2018 to June 2021 were retrospectively analyzed. The relationship between P/NR and clinical outcomes [early neurological deterioration (E-ND), HT, delayed ND (D-ND), and 3-mo outcomes] was scrutinized.

### *RESULTS*

Notable variables, such as age, diabetes, and stroke history, exhibited statistical disparities when comparing patients with and without E-ND, HT, D-ND, and 3-mo outcomes. P/NR prognostication revealed an optimal cutoff of 43.4 with a 60.3% sensitivity and a 52.5% specificity for 90-d outcomes. P/NR prognostic accuracy was statistically significant for 90-d outcomes [area under the curve (AUC) = 0.562], D-ND (AUC = 0.584), and HT (AUC = 0.607).

***CONCLUSION***
P/NR demonstrated an association with adverse 3-mo clinical outcomes, HT, and D-ND in AIS patients post-rtPA administration, indicating its potential as a predictive tool for complications and prognoses. This infers that a diminished P/NR may serve as a novel prognostic indicator, assisting clinicians in identifying AIS patients at elevated risk for unfavorable outcomes following rtPA therapy.

**Key Words:** Acute ischemic stroke; Platelet-to-neutrophil ratio; Prognosis; Hemorrhagic transformation; Recombinant tissue plasminogen activator; Thrombolysis; Clinical outcomes



**Core Tip:** The study explored the prognostic value of the platelet-to-neutrophil ratio (P/NR) in patients with acute ischemic stroke (AIS) who underwent thrombolysis with recombinant tissue plasminogen activator (rtPA). It aimed to determine if P/NR could predict hemorrhagic transformation and clinical outcomes following rtPA treatment. An optimal P/NR cutoff value was identified for predicting 90-d outcomes with moderate sensitivity and specificity. The study concluded that P/NR is associated with negative 3-mo outcomes, suggesting it could be a useful indicator for predicting risks post-rtPA treatment in AIS patients.

**Citation:** Chaiwisitkun A, Muengtaweepongsa S. Platelet-to-neutrophil ratio predicts hemorrhagic transformation and unfavorable outcomes in acute ischemic stroke with intravenous thrombolysis. *World J Exp Med* 2024; 14(3): 95540
**URL:** https://www.wjgnet.com/2220-315x/full/v14/i3/95540.htm
**DOI:** https://dx.doi.org/10.5493/wjem.v14.i3.95540

## INTRODUCTION

Stroke has perpetually maintained a predominant position, ranking within the top three, regarding disease burden over the past two decades, as assessed through disability-adjusted life-years (DALYs)[1,2]. The gravity of cerebral infarction, witnessed during acute ischemic stroke (AIS), bears a correlation with the disease's burden[3]. Ensuring successful AIS management proves pivotal in mitigating its associated burdens[4]. Cardioembolic and atherothrombotic strokes are the subtypes of ischemic infarct associated with the highest in-hospital mortality. The short-term prognosis for patients with these types of strokes is poorer compared to other ischemic stroke subtypes[5]. Despite the absence of a mortality reduction, intravenous thrombolysis employing recombinant tissue plasminogen activator (rtPA) has been substantiated to attenuate the burden in AIS[6]. Regrettably, merely 50% of patients manifest significantly favorable outcomes post intravenous rtPA administration[7]. Stroke survivors, perpetuating suboptimal outcomes subsequent to treatment, endure the remainder of their existence with disadvantageous DALYs[8]. A salient progenitor of unfavorable outcomes resides in complications attributed to intracranial hemorrhagic transformation (HT) in association with intravenous rtPA [9]. A multitude of predictive instruments for outcomes in AIS, following treatment with intravenous rtPA, have permeated clinical practice; nevertheless, the parameters encapsulated within each tool exhibit variance[10,11].

In the AIS pathogenesis, platelet activation and aggregation emerge as critical elements. Amidst pathological circumstances, an excessive activation and aggregation of platelets can precipitate thrombosis and vascular occlusion, thereby instigating ischemic stroke or heart disease[12]. A plethora of studies have authenticated a decrement in platelet count within the circulatory system of AIS patients, whereas the platelet distribution width and mean platelet volume experience an elevation[13]. The immune response is recognized as imperative in the pathological alterations observed in AIS. Ischemic and anoxic brain tissue instigates the infiltration of peripheral blood leukocytes into the afflicted area. Neutrophils, the initial cells to be recruited into the brain following a stroke, discharge inflammatory mediators within the ischemic brain area, exacerbate brain damage[14] and foster the incidence of ischemia by inducing thrombosis *via* various mechanisms, such as interfacing with platelets, coagulation factors, and discharging proteases[15]. The platelet-to-neutrophil ratio (P/NR) emerges as a novel biomarker that amalgamates platelets and neutrophil counts. Contrasting with singular platelet and neutrophil counts, P/NR mirrors the severity of both thrombosis and inflammation, elucidating the liaison between the two processes. Within the realm of stroke, a recent study posited that the level of P/NR upon admission is associated with the prognosis of AIS patients[16]. Furthermore, another study advocated that P/NR surpasses other complete blood count ratios in prognosticating an adverse outcome in AIS patients[17]. Within this retrospective study, our objective was to illustrate the clinical value of P/NR in prognosticating the outcome in AIS patients who have been treated with intravenous rtPA.

## MATERIALS AND METHODS

### Study population

This retrospective study was conducted utilizing data procured from Thammasat University Hospital (TUH). The patient cohort comprised individuals diagnosed with AIS who underwent intravenous thrombolysis treatment, specifically utilizing intravenous rtPA, in adherence to the TUH protocol between January 2018 and June 2021. A complete blood count (CBC) was mandated before confirming the decision to administer intravenous rtPA[18].

**Inclusion criteria:** Patients formally diagnosed with AIS who met the criteria for and consequently received intravenous rtPA treatment within 4.5 h of stroke onset, in line with the stroke fast-track criteria established by TUH. Age range between 18 years and 85 years.

**Exclusion criteria:** Patients with a history of infection or surgery within the preceding 2 wk. An underlying disease condition such as malignancy, rheumatoid arthritis, or connective tissue disease. Chronic liver disease (Child-Pugh Score > B). Chronic kidney disease (serum creatinine > 2.0 mg/dL). Prior abnormalities in platelet and white blood cell counts. Ultimately, 418 patients were incorporated into the study, forming the basis for subsequent analysis and findings.

### Ethical approval and methodological adherence

The Human Research Ethics Committee of Thammasat University (Medicine) bestowed approval for this study and granted a waiver for the requirement of informed consent, under approval number: 284/2564. All methodologies employed throughout the study adhered scrupulously to pertinent guidelines and regulations.

### Data collection

Data procurement entailed an assessment by proficient clinicians, predicated on clinical manifestations, to verify alignment with the diagnostic criteria for acute stroke. Stroke severity upon admission was gauged using the National Institute of Health Stroke Scale (NIHSS). To exclude hemorrhagic stroke, all patients underwent emergent imaging *via* computerized tomography (CT) scan or magnetic resonance imaging (MRI) prior to intravenous rtPA administration. Pre-rtPA intravenous CBC was obtained. Additionally, baseline clinical attributes, inclusive of alternative laboratory examinations within 24 h of admission [*e.g.*, fasting blood glucose and low-density lipoprotein (LDL)] and demographic data, were amassed for all patients[19].

### Evaluation standard

**Criteria were as follows:** Hypertension was characterized by recurrent systolic blood pressure readings ≥ 140 mmHg upon admission or antecedent hypertension history. Diabetes encapsulated either a prior diagnosis or admission with diabetes mellitus, and either fasting plasma glucose ≥ 126 mg/dL or HbA1C ≥ 6.5%. Atrial fibrillation (AF) required precedent AF episodes or admission-time AF electrocardiogram recordings. Hyperlipidemia entailed hyperlipidemia history or admission with dyslipidemia, and either LDL ≥ 100 mg/dL or triglyceride ≥ 150 mg/dL[20].

Infarct volume was calculated employing 418 cases examined *via* CT scans or MRI, utilizing the formula 0.5 × a × b × c (where a is the maximum longitudinal diameter, b is the maximum transverse diameter perpendicular to a, and c denotes 10 mm slices with infarction), with volumes < 5 $cm^3$ and ≥ 5 $cm^3$ defining small and large infarct volumes, respectively [21].

### Outcomes

Clinical outcomes encompassed early neurological deterioration (E-ND), HT, delayed ND (D-ND), and 3-mo poor outcomes. HT was delineated as any perceptible hemorrhage discerned on brain CT or MRI within 24 h post-thrombolysis, categorized *via* the Heidelberg Bleeding Classification[22]. E-ND was delineated as an augmentation of ≥ 4 points in NIHSS scores or death within 24 hours subsequent to intravenous thrombolysis. D-ND and 3-mo clinical outcomes were appraised utilizing the modified Rankin Scale (mRS), with D-ND and poor 3-mo outcomes defined as mRS scores of 3–6 at discharge (24 h to 7 d) and 3 mo post-onset, respectively.

### Statistical analysis

Analytical procedures were executed utilizing the Statistical Program for Social Sciences (SPSS), version 22.0 (IBM, West Grove, PA, United States). The Mann-Whitney U-test facilitated the evaluation of disparities between two groups for variables demonstrating a nonparametric distribution, while the Chi-square test was employed to discern variations between categorical variables. Continuous and categorical variables were depicted utilizing medians with interquartile ranges (IQR) and percentages, respectively. The prognostic impact of P/NR was appraised by employing the receiver operating characteristic (ROC) curve, with a *P*-value < 0.05 establishing statistical significance in all comparative group analyses.

## RESULTS

### Demographic and clinical overview of the study population

This investigation encompassed 418 patients, comprising 169 females (40.4%) and 249 males (59.6%), with a mean age of

64.5 years (range: 53-72 years) and a mean NIHSS score upon admission of 10 (IQR: 6-16). The predominant risk factors identified were hypertension (71.5%), hyperlipidemia (66%), and diabetes (33.7%). The mean time from stroke onset to intravenous rtPA administration was 170 min (IQR: 124-218 minutes). Laboratory findings included hemoglobin at 13.3 g/dL (IQR: 12.2-14.4) and white blood cell count at 8.4 × $10^9$/L (IQR: 6.81-10.51 × $10^9$/L), among other parameters. Antihypertensive therapy was the most prevalent current medication at 45.6%. Patient outcomes following intravenous rtPA at various time points (24 h, 24 h to 7 d, and 3 mo post-thrombolysis) were also analyzed. Of note, 24 (5.7%) exhibited E-ND, while 75 (18%) manifested HT within the initial 24 h following intravenous rtPA. Twelve patients (2.87%) died in the hospital. Of these, eight patients' deaths were due to neurologic complications.

### *Correlation between P/NR and clinical outcomes*

Eligible patients were stratified into groups according to the presence or absence of distinct clinical outcomes (E-ND, HT, D-ND, and 3-mo outcomes). In the E-ND assessment, statistically significant disparities were observed between groups with and without E-ND in terms of age, diabetes prevalence, current alcohol consumption, baseline blood glucose, and infarct volume, as further detailed. For instance, a statistically higher age was observed in the E-ND group (70 *vs* 64; $P$ = 0.045).

Differences were also evident when comparing patients with and without HT. Variables that demonstrated statistical variance encompassed previous stroke, stroke etiology, NIHSS upon admission, and LDL, among others. For instance, individuals without a prior stroke manifested a higher proportion of HT than those with a previous stroke (13% *vs* 4%; $P$ = 0.027).

Differences in clinical characteristics between the presence and absence of D-ND highlighted variables such as age, sex, and hypertension as statistically significant. For example, the D-ND group exhibited a higher mean age than the non-D-ND group (67 *vs* 60 years; $P$ < 0.001).

In distinguishing between favorable and unfavorable 3-mo clinical outcomes, statistically significant variations were found in variables such as age, sex, and hypertension. Notably, patients with poor 3-mo outcomes had a mean age of 69 years, contrasted with 60 years for those with favorable outcomes ($P$ < 0.001).

The ROC and area under the curve (AUC) analyses for P/NR in prognosticating 90-d outcomes post-ischemic stroke following intravenous thrombolysis revealed an optimal P/NR cutoff value of 43.4, with a 60.3% sensitivity and a 52.5% specificity, 32.86% (95%CI: 26.56-39.17) positive predictive value and a 77.45% (95%CI: 71.72-83.19) negative predictive value. P/NR demonstrated a statistically significant prognostic accuracy of 56.2% for 90-d outcomes (AUC = 0.562, 95%CI: 0.501-0.624, $P$ = 0.048).

ROC curves also provided prognostic insights for P/NR in relation to D-ND and HT post-ischemic stroke following intravenous thrombolysis. Notably, the P/NR offered a 58.4% accurate prognostication for D-ND (AUC = 0.584, 95%CI: 0.504-0.664, $P$ = 0.044), and a 60.7% accurate prognostication for HT (AUC = 0.607, 95%CI: 0.535-0.678, $P$ = 0.004).

These findings, along with further relevant data, are articulated within Figure 1, Tables 1, 2, 3, and 4, and Supplementary Table 1.

## DISCUSSION

The investigation discerned a pertinent association between the P/NR and adverse 3-mo clinical outcomes, HT, and D-ND in patients experiencing AIS post-intravenous administration of rtPA. A diminished P/NR was discernibly correlated with unfavorable outcomes, positioning P/NR as a potential novel prognostic indicator for complications and prognoses in the stated patient demographic.

Despite P/NR being a relatively nascent parameter within stroke research, preliminary studies indicate its potential predictive capabilities for outcomes in AIS. A study conducted by Jin *et al*[16] posited P/NR as a singularly protective predictor for 90-d outcomes in AIS, also noting that lower P/NR was concomitant with short-term adverse outcomes. In a parallel vein, Wang *et al*[23] associated post-rtPA P/NR with E-ND, HT, D-ND, and suboptimal 3-mo outcomes, echoing the predictive utility of lower P/NR for worse outcomes. Matsuoka *et al*[24] suggested P/NR could indicate a hypercoagulable state, potentially inducing ischemic stroke related to gastric cancer. While P/NR is corroborated in several studies as being associated with thrombosis, its relationship with prognoses of patients receiving intravenous rtPA has not been comprehensively explored[25].

Insights from existing research elucidate that platelet-neutrophil interactions play a pivotal role in inflammation and thrombosis, particularly during AIS[26]. The intravascular thrombosis and ensuing inflammatory response precipitate a reduction in platelets and a surge in neutrophils, cumulatively resulting in diminished P/NR levels. Accordingly, a rational deduction can be drawn that low P/NR levels are independently associated with adverse AIS outcomes.

Moreover, considering the thrombolysis combination, symptomatic intracranial hemorrhage potentially exacerbates symptoms. A plethora of studies have demonstrated that a synergy of decreased platelets and elevated neutrophils can contribute to symptomatic intracranial hemorrhage[27-29]. Gensicke *et al*[30] provided insights into the mechanistic link between poor outcomes and neutrophils, elucidating that the latter disrupts the blood-brain barrier by liberating matrix metalloproteinase-9 and augmenting reactive oxygen and nitrogen species[29,30]. The conglomeration of these findings substantiates the hypothesis that P/NR may serve as a viable prognostic predictor for patient outcomes.

The present study not only benefits from an ample sample size, ensuring enhanced reliability and persuasive power of results, but also distinguishes itself as one of the few concentrating on the correlation between P/NR and prognosis in AIS patients treated with intravenous rtPA. Nonetheless, the implications of the findings should be interpreted considering several limitations, including the retrospective nature of the study and potential unconsidered confounders.

**Table 1 Clinical characteristics of the study population**

| Characteristics | Number |
|---|---|
| Age in yr | 64.5 (53-72) |
| Sex | |
| Male | 249 (59.6) |
| Female | 169 (40.4) |
| Risk factor | |
| Hypertension | 299 (71.5) |
| Dyslipidemia | 276 (66) |
| Diabetes mellitus | 141 (33.7) |
| Atrial fibrillation/atrial flutter | 102 (24.4) |
| Old stroke | 47 (11.2) |
| Current smoking | 73 (17.5) |
| Current alcohol drinking | 42 (10) |
| Etiology | |
| Other determined or undetermined | 177 (42.3) |
| Cardioembolic | 102 (24.4) |
| Small-artery occlusion | 94 (22.5) |
| Large-artery atherosclerosis | 42 (10) |
| Medication before stroke onset | |
| Antihypertensive therapy | 202 (48.3) |
| Antiplatelet therapy | 81 (18.3) |
| Hypoglycemic therapy | 121 (27.3) |
| Time for stroke onset to intravenous rtPA infusion in min | 170.05 (124-218.25) |
| Infarct volume in mL | 3.27 (0.58-24.24) |
| Hemorrhagic transformation | |
| No | 343 (82) |
| Yes | 75 (82) |
| PH1 | 29 (82) |
| PH2 | 27 (6.1) |
| HI1 | 12 (2.7) |
| HI2 | 7 (1.6) |

Data are *n* (%)/median ($25^{th}$-$75^{th}$ percentiles). HI: Hemorrhagic infarct; PH: Parenchymal hemorrhage; rtPA: Recombinant tissue plasminogen activator.

Furthermore, the solitary hospital data source may induce selection bias, P/NR levels were measured only at a single time point (upon admission), and no dynamic monitoring was conducted. Also, numerous pre-existing conditions and infections that influence inflammation could potentially impact the P/NR ratio.

## CONCLUSION

In recapitulation, the findings elucidate that P/NR demonstrates an independent association with unfavorable 3-mo outcomes (mRS ≥ 3), HT, and D-ND. A lower P/NR level could potentially serve as a predictor for adverse outcomes, thereby offering a novel parameter that neurologists might employ for prognosticating stroke outcomes in clinical settings. Prospective studies encompassing larger sample sizes and dynamic P/NR monitoring are requisite for further exploration.

**Table 2 Clinical characteristics of the study population (continued)**

| Characteristics | Number |
|---|---|
| Baseline blood glucose in mg% | 110 (96-141) |
| Laboratory tests | |
| Hb | 13.3 (12.2-14.4) |
| WBC as $10^9$/L | 8.4 (6.81-10.51) |
| Platelets as $10^9$/L | 227 (192-278) |
| Neutrophil as $10^9$/L | 5.14 (3.8-7.06) |
| Lymphocyte as $10^9$/L | 1.93 (1.36-2.7) |
| P/NR | 43.73 (32.0-59.04) |
| PLR | 115.33 (87.17-170.64) |
| NLR | 2.56 (1.61-4.52) |
| PWR | 27.07 (21.4-33.99) |
| LDL | 114.5 (89-143) |
| NIHSS on admission | 10 (6-16) |
| NIHSS score on discharge date (day 1-7) | 5 (1-10) |
| Outcome events | |
| Increase NIHSS from baseline or death within 7 d after IV rt-PA | |
| Poor outcome (≥ 4 score) | 24 (5.7) |
| Good outcome (< 4 score) | 394 (94.3) |
| mRS on admission | 5 (3.75-5) |
| Poor outcome (3-6) | 348 (83.3) |
| Good outcome (0-2) | 70 (16.7) |
| mRS on discharge date (day 1-7) | 3 (1-4) |
| Poor outcome (3-6) | 236 (56.5) |
| Good outcome (0-2) | 182 (43.5) |
| mRS at 3 mo | 2 (0-4) |
| Poor outcome (3-6) | 168 (40.2) |
| Good outcome (0-2) | 250 (59.8) |

Data are *n* (%)/median ($25^{th}$-$75^{th}$ percentiles). Hb: Hemoglobin; LDL: Low density lipoprotein; mRS: Modified Rankin Scale; NIHSS: National Institute of Health Stroke Scale; NLR: Neutrophil-to-lymphocyte ratio; P/NR: Platelet-to-neutrophil ratio; PLR: Platelet-to-lymphocyte ratio; WBC: White blood cell.

**Table 3 Clinical characteristics of patients according to presence/absence of early neurological deterioration and hemorrhagic transformation after intravenous recombinant tissue plasminogen activator treatment**

| Variables | Total, *n* = 418 | No E-ND, *n* = 394 | E-ND, *n* = 24 | *P* value | No HT, *n* = 342 | HT, *n* = 75 | *P* value |
|---|---|---|---|---|---|---|---|
| Age in yr | 64.5 (53-72) | 64 (52-71) | 70 (58.75-77.25) | 0.045[1] | 65 (53-72) | 64 (52-74) | 0.939[1] |
| Sex | | | | | | | |
| Male | 249 (59.6) | 237 (60.2) | 12 (50) | 0.325[2] | 206 (60.2) | 42 (56.0) | 0.4992 |
| Female | 169 (40.4) | 157 (39.8) | 12 (50) | | 136 (39.8) | 33 (44.0) | |
| Risk factor | | | | | | | |
| Hypertension | 299 (71.5) | 279 (71.2) | 20 (83.3) | 0.198[2] | 245 (72.1) | 53 (70.7) | 0.808[2] |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Dyslipidemia | 276 (66.0) | 261 (66.9) | 15 (62.5) | 0.655[2] | 223 (65.8) | 52 (70.3) | 0.458[2] |
| Diabetes mellitus | 141 (33.7) | 126 (32.1) | 15 (62.5) | 0.002[2] | 114 (33.4) | 26 (34.7) | 0.838[2] |
| Atrial fibrillation/ atrial flutter | 102 (24.4) | 97 (24.6) | 5 (20.8) | 0.675[2] | 81 (23.7) | 21 (28.0) | 0.431[2] |
| Old stroke | 47 (11.2) | 44 (11.3) | 3 (12.5) | 0.852[2] | 44 (13.0) | 3 (4.0) | 0.027[2] |
| Current smoking | 73 (17.5) | 70 (82.4) | 3 (100) | 0.424[2] | 62 (83.8) | 10 (76.9) | 0.546[2] |
| Current alcohol drinking | 42 (10.0) | 42 (91.3) | 0 (0) | 0.003[2] | 37 (90.2) | 5 (83.3) | 0.608[2] |
| Etiology | | | | | | | |
| Other determined or undetermined | 177 (42.3) | 161 (41.2) | 16 (66.7) | 0.005[2] | 143 (42.1) | 34 (45.3) | < 0.001[2] |
| Cardioembolic | 102 (24.4) | 100 (25.6) | 2 (8.3) | | 73 (21.5) | 29 (38.7) | |
| Small-artery occlusion | 94 (22.5) | 93 (23.8) | 1 (4.2) | | 91 (26.8) | 3 (4.0) | |
| Large-artery atherosclerosis | 42 (10) | 37 (9.5) | 5 (20.8) | | 33 (9.7) | 9 (12.0) | |
| Medication | | | | | | | |
| Antihypertensive therapy | 202 (45.6) | 75 (19.0) | 6 (25.0) | 0.473[2] | 165 (48.2) | 37 (49.3) | 0.864[2] |
| Antiplatelet therapy | 81 (18.3) | 191 (48.5) | 11 (45.8) | 0.801[2] | 63 (18.4) | 18 (24.0) | 0.269[2] |
| Hypoglycemic therapy | 121 (27.3) | 111 (28.2) | 10 (41.7) | 0.16[2] | 96 (28.2) | 25 (33.3) | 0.371[2] |
| Infarct volume in mL | 3.27 (0.58-24.24) | 2.76 (0.45-20.25) | 34.42 (5.57-303.93) | < 0.001[1] | 2.67 (0.43-16.88) | 13.99 (1.11-73.07) | 0.389[1] |
| Time for stroke onset to intravenous rtPA infusion in min | 170.05 (124-218.25) | 170 (122-217) | 180.5 (147.5-232.25) | 0.157[1] | 172 (124.85-218.25) | 160 (124-219) | 0.516[1] |

[1]Mann Whitney *U* test.
[2]$\chi^2$ test.
Data are *n* (%)/median (25$^{th}$-75$^{th}$ percentiles). E-ND: Early neurological deterioration; HT: Hemorrhagic transformation; rtPA: Recombinant tissue plasminogen activator.

**Table 4 Clinical characteristics of patients according to presence/absence of delayed neurological deterioration and 3-mo outcome after intravenous recombinant tissue plasminogen activator treatment**

| Variables | Total, *n* = 418 | No D-ND, *n* = 182 | D-ND, *n* = 236 | *P* value | Good 3-months, *n* = 250 | Poor 3-months, *n* = 168 | *P* value |
|---|---|---|---|---|---|---|---|
| Age in yr | 64.5 (53-72) | 60 (49-67.25) | 67 (58-76.75) | < 0.001[1] | 60 (50-68) | 69 (61-75) | < 0.001[1] |
| Sex | | | | | | | |
| Male | 249 (59.6) | 120 (65.9) | 129 (54.7) | 0.02[2] | 162 (64.8) | 87 (51.8) | 0.008[2] |
| Female | 169 (40.4) | 62 (34.1) | 107 (45.3) | | 88 (35.2) | 81 (48.2) | |
| Risk factor | | | | | | | |
| Hypertension | 299 (71.5) | 114 (62.6) | 185 (79.1) | < 0.001[2] | 163 (65.2) | 136 (81.9) | < 0.001[2] |
| Dyslipidemia | 276 (66.0) | 113 (62.8) | 163 (69.7) | 0.141[2] | 162 (65.3) | 114 (68.7) | 0.478[2] |
| Diabetes Mellitus | 141 (33.7) | 55 (30.4) | 86 (36.4) | 0.195[2] | 73 (29.3) | 68 (40.5) | 0.018[2] |
| Atrial fibrillation/atrial flutter | 102 (24.4) | 40 (22.0) | 62 (26.3) | 0.311[2] | 53 (21.2) | 49 (29.2) | 0.063[2] |
| Old stroke | 47 (11.2) | 16 (8.8) | 31 (13.2) | 0.16[2] | 24 (9.7) | 23 (13.7) | 0.21[2] |
| Current smoking | 73 (17.5) | 38 (86.4) | 35 (79.5) | 0.395[2] | 52 (85.2) | 21 (77.8) | 0.39[2] |
| Current alcohol drinking | 42 (10.0) | 20 (95.2) | 22 (84.6) | 0.24[2] | 30 (88.2) | 12 (92.3) | 0.685[2] |
| Etiology | | | | | | | |
| Other determined or undetermined | 177 (42.3) | 69 (38.3) | 108 (46.0) | 0.004[2] | 93 (37.5) | 84 (50.3) | 0.002[2] |
| Cardioembolic | 102 (24.4) | 51 (28.3) | 51 (21.7) | | 70 (28.2) | 32 (19.2) | |
| Small-artery occlusion | 94 (22.5) | 50 (27.8) | 44 (18.7) | | 66 (26.6) | 28 (16.8) | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Large-artery atherosclerosis | 42(10) | 10 (5.6) | 32 (13.6) | | 19 (7.7) | 23 (13.8) | |
| Medication | | | | | | | |
| Antihypertensive therapy | 202 (45.6) | 81 (44.5) | 121 (51.3) | 0.17[2] | 111 (44.4) | 91 (54.2) | 0.05[2] |
| Antiplatelet therapy | 81 (18.3) | 31 (17.0) | 50 (21.2) | 0.287[2] | 44 (17.6) | 37 (22.0) | 0.262[2] |
| Hypoglycemic therapy | 121 (27.3) | 53 (29.1) | 68 (28.9) | 0.967[2] | 71 (28.5) | 50 (29.8) | 0.783[2] |
| Infarct volume in mL | 3.27 (0.58-24.24) | 1.16 (0.05-7.88) | 7.61 (1.53-63.41) | 0.36[1] | 1.46 (0.13-7.93) | 16.62 (2.51-103.09) | 0.013[1] |
| Time for stroke onset to intravenous rtPA infusion in min | 170.05 (124-218.25) | 182 (0-125) | 167.5 (120-219.5) | 0.528[1] | 173.5 (125-218) | 166.5 (120-220) | 0.306[1] |

[1]Mann Whitney *U* test.
[2]$\chi^2$ test.
Data are *n* (%)/median ($25^{th}$-$75^{th}$ percentiles). D-ND: Delayed neurological deterioration; rtPA: Recombinant tissue plasminogen activator.

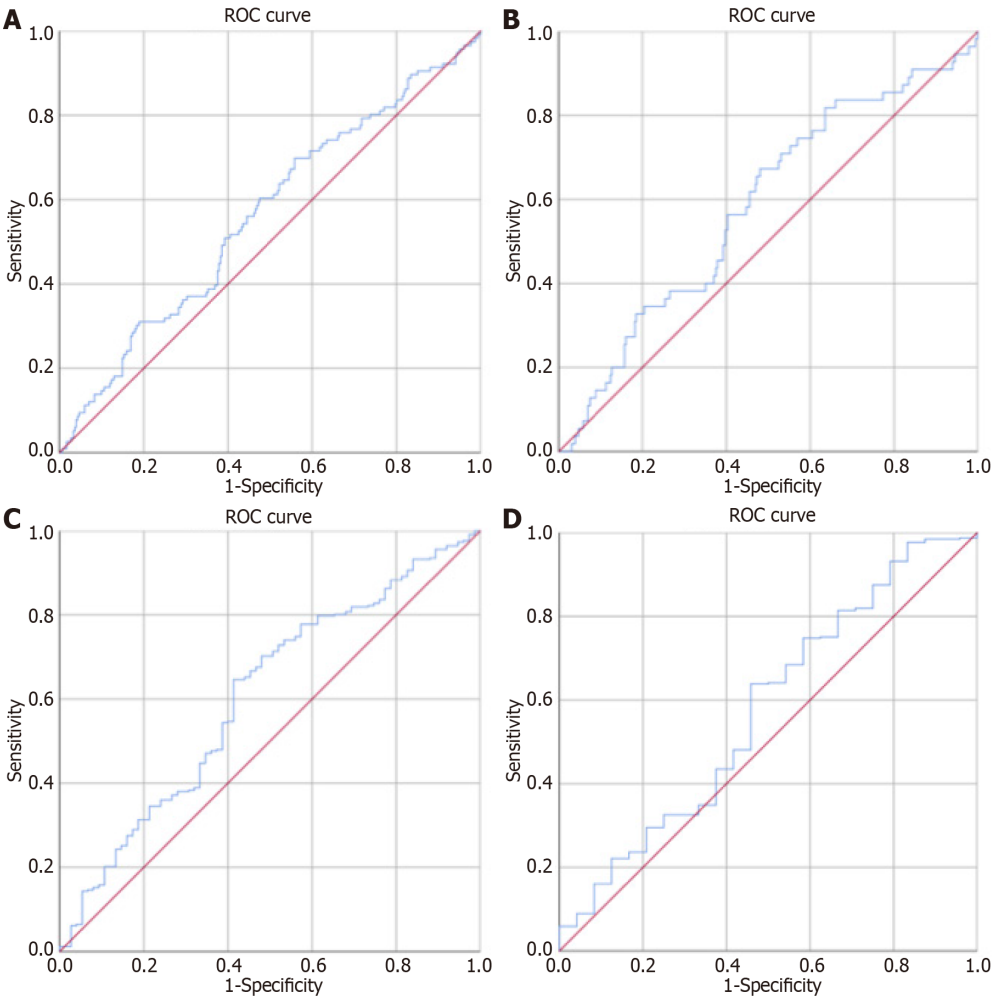


**Figure 1 Receiver operating characteristic curve.** A: Receiver operating characteristic curve (ROC) of platelets to neutrophil ratio (P/NR) for predicting 90-d outcome in acute ischemic stroke after intravenous recombinant tissue plasminogen activator (rtPA); B: ROC of P/NR for predicting delay neurological deterioration in acute ischemic stroke after intravenous rtPA; C: ROC of P/NR for predicting hemorrhagic transformation in acute ischemic stroke after intravenous rtPA; D: ROC of P/NR for predicting early neurological deterioration in acute ischemic stroke after intravenous rtPA.

## ACKNOWLEDGEMENTS

The authors wish to thank Prof. Paskorn Sritipsukho from the Center of Excellence in Applied Epidemiology, Thammasat University, for his help with biostatistical analysis.

## FOOTNOTES

**Author contributions:** Chaiwisitkun A and Muengtaweepongsa S were responsible for the conceptualization and design of the study; Muengtaweepongsa S was responsible for the formal analysis; Chaiwisitkun A and Muengtaweepongsa S were responsible for the investigation; Chaiwisitkun A was responsible for the original draft preparation; Muengtaweepongsa S was responsible for validation, review, and editing.

**Institutional review board statement:** The Human Research Ethics Committee of Thammasat University (Medicine) bestowed approval for this study and granted a waiver for the requirement of informed consent, under approval number 284/2564. All methodologies employed throughout the study adhered scrupulously to pertinent guidelines and regulations.

**Informed consent statement:** The Human Research Ethics Committee of Thammasat University (Medicine) bestowed approval for this study and granted a waiver for the requirement of informed consent, under approval number 284/2564.

**Conflict-of-interest statement:** The author(s) declare no competing interests.

**Data sharing statement:** The datasets generated and/or analyzed during the current study are available in the Research Gate repository, DOI: 10.13140/RG.2.2.25908.73606.



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**S-Editor:** Liu H
**L-Editor:** Filipodia
**P-Editor:** Wang WB

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World Journal of Experimental Medicine

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*World J Exp Med* 2024 September 20; 14(3): 93869

DOI: 10.5493/wjem.v14.i3.93869

ISSN 2220-315x (online)

ORIGINAL ARTICLE

Clinical and Translational Research

# Interaction between tumor stage and age on survival outcomes of patients with anaplastic thyroid cancer

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**Specialty type:** Oncology

**Provenance and peer review:** Invited article; Externally peer reviewed.

**Peer-review model:** Single blind

**Peer-review report's classification**

**Scientific Quality:** Grade B, Grade C

**Novelty:** Grade B, Grade C

**Creativity or Innovation:** Grade B, Grade C

**Scientific Significance:** Grade B, Grade C

**P-Reviewer:** Sahin TT; Ueda H

**Received:** March 7, 2024

**Revised:** June 13, 2024

**Accepted:** June 25, 2024

**Published online:** September 20, 2024

**Processing time:** 174 Days and 23.9 Hours

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## Abstract

### *BACKGROUND*

Anaplastic thyroid cancer (ATC) is an aggressive, rare malignancy associated with rapid growth and metastasis, and a very poor prognosis. We investigated the clinical characteristics, survival outcomes and independent prognostic factors associated with anaplastic thyroid cancer.

### *AIM*

To assess to what extent the interaction between age and tumor stage affects mortality.

***METHODS***
A total of 622 patients diagnosed with anaplastic thyroid cancer, between 2010 and 2017 were enrolled in our study by retrieving data from the Surveillance, Epidemiology and End Results (SEER) database. We analyzed demographics, clinical characteristics, overall mortality (OM) and cancer specific mortality (CSM) of ATC. Variables with a *P* value < 0.1 were incorporated into the multivariate cox model to determine the independent prognostic factors. Furthermore, we analyzed the interaction between age and tumor stage on mortality.

***RESULTS***
In the multivariate analyses, the divorced/separated population had a lower OM [hazard ratio (HR) = 0.63, 95%CI: 0.42-0.94, *P* < 0.05] and CSM (HR = 0.61, 95%CI: 0.40-0.92, *P* < 0.05). OM was higher in tumors with direct extension only (HR = 6.26, 95%CI: 1.29-30.42, *P* < 0.05) and tumors with distant spread (HR = 5.73, 95%CI: 1.34-24.51, *P* < 0.05). CSM was also higher in tumors with direct extension (HR = 5.05, 95%CI: 1.05-24.19, *P* < 0.05) and tumors with distant spread (HR = 4.57, 95%CI: 1.08-19.29, *P* < 0.05). Mortality was not adversely affected by lymph node involvement. OM was lower in patients who received radiation (HR = 0.66, 95%CI: 0.53-0.83, *P* < 0.01), chemotherapy (HR = 0.63, 95%CI: 0.50-0.79, *P* < 0.01) or surgery (HR = 0.53, 95%CI: 0.43-0.66, *P* < 0.01). CSM was also lower in patient who received radiation (HR = 0.64, 95%CI: 0.51-0.81, *P* < 0.01), chemotherapy (HR = 0.62, 95%CI: 0.50-0.78, *P* < 0.01) or surgery (HR = 0.51, 95%CI: 0.41-0.63, *P* < 0.01). There was no significant interaction between age and tumor stage that affected mortality.

***CONCLUSION***
In this large US SEER database retrospective study, we found the mortality to be higher in advanced stage tumors with direct extension and distant metastasis. However, patients who received aggressive therapy showed a better overall survival. The aim of our study is to emphasize the importance of detecting ATC at an early stage and provide aggressive therapy to these patients. Since advanced stage ATC is associated with a dismal prognosis, we emphasize the need for randomized control trials and development of novel therapies that will be used to treat ATC.

**Key Words:** Undifferentiated thyroid cancer; Survival outcome; Prognosis; Metastasis; Clinical trials; Interaction



**Core Tip:** Anaplastic thyroid cancer (ATC) represents a rare and highly aggressive subtype of thyroid cancer. Due to its rarity, limited information exists regarding the survival outcomes of patients with ATC. This study aims to provide the most current and comprehensive analysis of survival outcomes among ATC patients, with a specific emphasis on the interaction between two independent prognostic factors. Our findings indicate higher mortality rates in cases of advanced-stage tumors with direct extension and distant metastasis. Nevertheless, patients who underwent aggressive therapy exhibited improved overall survival rates.

**Citation:** Bangolo AI, Auda A, Bukasa-Kakamba J, Bhakta N, Dey S, lilhori A, Reddy G, Alqinai B, Sidiqui A, Sekhon I, Khatiashvili B, Abbas I, Kunnel S, Jarri A, Martinez E, Daoud D, Gupta I, Gompa H, Pender S, Aljaberi D, Aljanaahi H, Kunnel SS, Xiao Y, Jung Y, Nagpaul S, Naz A, Mallela T, Maung PM, Khalaf IY, Kim S, Alrestom R, Gajera A, Alkealy H, Kansal D, Dhall S, Satheesha S, Weissman S, Fwelo P. Interaction between tumor stage and age on survival outcomes of patients with anaplastic thyroid cancer. *World J Exp Med* 2024; 14(3): 93869
**URL:** https://www.wjgnet.com/2220-315x/full/v14/i3/93869.htm
**DOI:** https://dx.doi.org/10.5493/wjem.v14.i3.93869

## INTRODUCTION

Anaplastic thyroid cancer (ATC) is an aggressive and rare malignancy of the thyroid gland, accounting for approximately 1%-2% of all thyroid cancers[1]. It is characterized by rapid growth, early metastasis, and a dismal prognosis, with a median survival time of less than six months[2]. Over the past decade, significant advancements in our understanding of ATC's pathogenesis and treatment have emerged, shedding light on the critical factors influencing patient outcomes. The interaction between tumor stage and patient age are among these factors that have increased the attention of the medical community. This introduction aims to provide a comprehensive overview of the dynamic interplay between tumor stage and age and its impact on the survival outcomes of patients with ATC.

Thyroid cancer, in general, has been on the rise in recent years, with ATC representing the most aggressive form. Age has long been recognized as a crucial determinant in cancer development and progression, with distinct patterns observed across various cancer types. For ATC, it is imperative to explore how age influences the disease's presentation, progression, and survival outcomes, as it may offer invaluable insights into personalized treatment strategies. Several

studies have highlighted age as an independent prognostic factor for ATC patients, with older individuals often experiencing poorer survival rates[3]. Age-related disparities in tumor biology, immune response, and treatment tolerance contribute to these findings. Recent research suggests that age-related molecular alterations within ATC tumors may influence disease aggressiveness. These age-related genomic differences can affect tumor growth, response to therapy, and overall survival, further emphasizing the importance of considering age in the management of ATC[4].

Tumor stage at diagnosis remains a critical predictor of survival in ATC patients. The tumor, node, metastasis staging system provides valuable insights into disease severity[5]. However, it is essential to investigate how age modifies the prognostic significance of tumor stage in ATC. Age-related variations in clinical presentation and diagnostic delays are well-documented in ATC[6]. Older patients often present with more advanced disease due to less aggressive diagnostic workup, potentially confounding the association between tumor stage and age.

The management of ATC poses unique challenges in older patients, who may have comorbidities and reduced functional status. These factors can impact treatment choices and overall survival[7]. Over the past decade, novel treatment approaches, such as targeted therapies and immunotherapies, have shown promise in ATC[8]. However, the efficacy and safety of these treatments may vary with age, necessitating a nuanced assessment of their impact on survival outcomes. Combining surgery, radiation, and systemic therapies is often the cornerstone of ATC treatment. Understanding how age influences treatment response and tolerance is crucial in optimizing therapeutic strategies[9,10]. While age is typically considered a prognostic factor for various malignancies, thyroid cancer stands out due to its unique incorporation of age as a staging variable in the assessment of prognosis. Age can significantly impact treatment decision-making, with older patients often prioritizing quality of life over aggressive interventions. Shared decision-making between patients, families, and healthcare providers is paramount[11].

With the rapid evolution of precision medicine, ongoing research aims to identify age-specific biomarkers and therapeutic targets for ATC. Tailored treatment approaches may offer improved survival outcomes for both younger and older patients. International collaborations and data-sharing initiatives have facilitated the pooling of data from various centers, enabling the examination of large cohorts of ATC patients across different age groups[12].

## MATERIALS AND METHODS

### Study design

A retrospective cohort study was carried out on patients with ATC using data from the SEER research database. This database, managed by the United States National Cancer Institute, is renowned for its comprehensive cancer-related dataset. It encompasses 18 population-based cancer registries, collectively known as SEER 18, which compile data on cancer incidence, clinicopathological characteristics of patients, and survival rates. Covering approximately 28% of the United States population, the SEER 18 database is a significant and authoritative resource for cancer research[13].

### Data selection

**Inclusion criteria:** We included all patients diagnosed with Hepatosplenic T-Cell Lymphoma between 2010 and 2017 in our cohort, identified from the SEER database using criteria related to primary site and histological type. Data for these patients were extracted from the SEER database using the specified International Classification of Diseases (ICD)-9, ICD-10, and/or ICD-O-3 codes.

**Exclusion criteria:** Patients with unknown age at diagnosis, race, or stage of ATC were excluded from the study.

### Study variables

**Main exposure:** With the exception of the year of diagnosis, all variables within this cohort were utilized as primary predictors of prognosis.

### Outcomes

Overall mortality was defined as patients who died from any cause by the end of the study being categorized as "yes", while those who did not were categorized as "no".

Cancer-specific mortality referred to patients who died from ATC at the end of the study being categorized as "yes", while those who died from other causes were classified as "no".

### Survival months

Regarding overall mortality, survival time was computed from the date of diagnosis to either the date of death or the last follow-up date (December 31, 2017), as documented in the SEER registry.

Regarding cancer-specific mortality, survival time was determined from the date of diagnosis to either the date of death due to ATC or the last follow-up date, as documented in the SEER registry.

### Sociodemographic and tumor characteristics

The following variables were extracted: Age at diagnosis, gender, race (White, Black, and others), ethnicity (Non-Hispanic and Hispanic), stage at diagnosis (localized, regional, and distant), geographic residential area, annual income, marital status, year of diagnosis, surgery, radiation and chemotherapy.

### *Statistical analysis*

The Cox proportional hazards regression model relies on the assumption that hazard rates remain proportional over time. Variables with a significance level of < 0.1 in the univariate Cox regression model were included in the multivariate Cox proportional analysis to identify independent prognostic factors associated with overall mortality (OM) and cancer-specific mortality (CSM), where a hazard ratio (HR) > 1 indicates adverse prognostic factors. All tests were two-sided, with a confidence interval set at 95%, and a *P* value < 0.05 considered statistically significant. Statistical analyses were conducted using STATA 18.0 software.

## RESULTS

We conducted our study with a cohort of ATC patients, comprising a total of 622 individuals. Table 1 provides a comprehensive overview of the baseline characteristics of our study population. In our cohort, 58.84% were female, and 41.16% were male. Age distribution at diagnosis revealed that 20.58% fell within the 00-59 age group, 54.98% within the 60-79 age group, and 24.44% were aged 80 and above. Marital status varied, with 55.31% being married, 16.40% single, 6.75% divorced/separated, and 21.54% widowed. Tumor stage distribution indicated that a significant proportion (74.44%) of patients presented with distant metastasis, while 5.47% had localized tumors. In terms of race, the majority were non-Hispanic white (61.09%), followed by hispanic (18.01%), other (13.34%), and non-Hispanic black (7.56%). Living area analysis showed 58.68% in counties with a population of 1 million persons, 24.28% in areas with 250000 to 1 million persons, 5.14% in areas with 250000 persons, 6.59% in nonmetropolitan counties adjacent to a metropolitan area, and 5.31% in nonmetropolitan counties not adjacent to a metropolitan area. Income per year exhibited a diverse distribution, with 4.66% earning less than $45000, 35.85% earning $75000 and above, and varying percentages in the income brackets between. Treatment modalities varied, with 42.93% not receiving radiation, 55.14% not receiving chemotherapy, and 56.43% not undergoing surgery. The study spanned across different years of diagnosis, ranging from 12.06% in 2010 to 15.92% in 2017. These baseline characteristics provide a detailed snapshot of the demographic and clinicopathologic profile of our ATC patient cohort.

Table 2 illustrates the crude analysis of factors associated with all-cause mortality (OM) and CSM among United States patients diagnosed with ATC between 2010 and 2017. Age at diagnosis demonstrated significant associations, with higher OM and CRM observed in older age groups: 60-79 years (OM HR = 1.38, 95%CI: 1.10-1.74; CRM HR = 1.29, 95%CI: 1.02-1.64) and 80 + years (OM HR = 1.64, 95%CI: 1.24-2.16; CSM HR = 1.16, 95%CI: 1.19-2.08). Tumor stage also showed significant associations, with higher HRs for more advanced stages, particularly in tumors with local spread (OM HR = 2.12, 95%CI 1.22-3.66, $P < 0.01$; CSM HR = 2.07, 95%CI: 1.16-3.66, $P < 0.01$) and tumors with distant spread (OM HR = 3.24, 95%CI: 2.05-5.12; CSM HR = 3.25, 95%CI: 2.01-5.24). Furthermore, treatment modalities like radiation, chemotherapy, and surgery demonstrated significant associations with lower OM and CRM. Radiation (OM HR = 0.61, 95%CI: 0.50-0.74; CRM HR = 0.59, 95%CI: 0.49-0.72), chemotherapy (OM HR = 0.61, 95%CI: 0.50-0.73; CRM HR = 0.60, 95%CI: 0.49-0.72), and surgery (OM HR = 0.50, 95%CI: 0.41-0.59; CRM HR = 0.47, 95%CI: 0.39-0.58) were all significantly associated with lower OM and CRM. Associations marked with $^{a}P < 0.05$ and $^{b}P < 0.01$ indicate statistical significance at the respective levels. Confidence intervals are reported at the 95% level.

In Table 3, multivariate analyses, age did not demonstrate an adverse effect on mortality outcomes. However, the divorced/separated population exhibited a significantly lower risk of overall mortality (HR = 0.63, 95%CI: 0.42-0.94, $P < 0.05$) and cancer-specific mortality (HR = 0.61, 95%CI: 0.40-0.92, $P < 0.05$). Conversely, overall mortality was higher in tumors with direct extension only (HR = 6.26, 95%CI: 1.29-30.42, $P < 0.05$) and those with distant spread (HR = 5.73, 95%CI: 1.34-24.51, $P < 0.05$). Similarly, cancer-specific mortality was elevated in tumors with direct extension (HR = 5.05, 95%CI: 1.05-24.19, $P < 0.05$) and distant spread (HR = 4.57, 95%CI: 1.08-19.29, $P < 0.05$). However, mortality was not adversely affected by lymph node involvement. Moreover, patients who received radiation (HR = 0.66, 95%CI: 0.53-0.83, $P < 0.01$), chemotherapy (HR = 0.63, 95%CI: 0.50-0.79, $P < 0.01$), or surgery (HR = 0.53, 95%CI: 0.43-0.66, $P < 0.01$) had significantly lower risks of overall mortality. Similarly, cancer-specific mortality was reduced in patients who received radiation (HR = 0.64, 95%CI: 0.51-0.81, $P < 0.01$), chemotherapy (HR = 0.62, 95%CI: 0.50-0.78, $P < 0.01$), or surgery (HR = 0.51, 95%CI: 0.41-0.63, $P < 0.01$).

In Table 4's multivariate Cox proportional hazard regression analyses considering the interaction between tumor stage and age, several notable findings emerge regarding all-cause mortality (OM) and ATC-related mortality (CSM). In terms of specific interactions, there is no interaction between tumor stage and age significantly influencing mortality outcomes. However, it's important to note that the confidence intervals for many of these estimates are wide, indicating uncertainty in the estimates due to small sample sizes within some subgroups. Therefore, further investigation with larger datasets may be warranted to better understand the potential interactions between tumor stage and age on mortality outcomes in ATC patients.

## DISCUSSION

In this large retrospective study using the SEER database, we found a female and non-Hispanic white predominance, most patients were diagnosed between 60 and 79 and at an advanced stage. There was almost an equal distribution between patients that received therapy and those that did not. Advanced age and advanced stage were associated with increased OM and CSM and all treatment modalities were associated with a lower OM and CSM, in the univariate

**Table 1 Demographic and clinicopathologic characteristics of United States patients diagnosed with anaplastic thyroid cancer between 2010 and 2017**

| Characteristics | *n* | Percent |
|---|---|---|
| Gender | | |
| Female | 366 | 58.84 |
| Male | 256 | 41.16 |
| Age at diagnosis, year old | | |
| 00-59 | 128 | 20.58 |
| 60-79 | 342 | 54.98 |
| 80 + | 152 | 24.44 |
| Marital status | | |
| Married | 344 | 55.31 |
| Single | 102 | 16.40 |
| Divorced/separated | 42 | 6.75 |
| Widowed | 134 | 21.54 |
| Tumor stage | | |
| Localized | 34 | 5.47 |
| Regional by direct extension only | 47 | 7.56 |
| Regional lymph nodes involved only | 25 | 4.02 |
| Regional by both direct extension and lymph node involvement | 53 | 8.52 |
| Distant | 463 | 74.44 |
| Race | | |
| Non-Hispanic white | 380 | 61.09 |
| Non-Hispanic black | 47 | 7.56 |
| Hispanic | 112 | 18.01 |
| Other | 83 | 13.34 |
| Living area | | |
| Counties in metropolitan areas of 1 million persons | 365 | 58.68 |
| Counties in metropolitan areas of 250000 to 1 million persons | 151 | 24.28 |
| Counties in metropolitan areas of 250000 persons | 32 | 5.14 |
| Nonmetropolitan counties adjacent to a metropolitan area | 41 | 6.59 |
| Nonmetropolitan counties not adjacent to a metropolitan area | 33 | 5.31 |
| Income per year | | |
| $ < $45000 | 29 | 4.66 |
| $45000-54999 | 69 | 11.09 |
| $55000-64999 | 127 | 20.42 |
| $65000-74999 | 174 | 27.97 |
| $75000 + | 223 | 35.85 |
| Radiation | | |
| No | 267 | 42.93 |
| Yes | 355 | 57.07 |
| Chemotherapy | | |
| No | 343 | 55.14 |

| | | |
|---|---|---|
| Yes | 279 | 44.86 |
| Surgery | | |
| No | 351 | 56.43 |
| Yes | 271 | 43.57 |
| Year of diagnosis | | |
| 2010 | 75 | 12.06 |
| 2011 | 67 | 10.77 |
| 2012 | 64 | 10.29 |
| 2013 | 68 | 10.93 |
| 2014 | 74 | 11.90 |
| 2015 | 79 | 12.70 |
| 2016 | 96 | 15.43 |
| 2017 | 99 | 15.92 |

analysis. In the univariate analysis however, age did not affect mortality and interestingly, distant metastasis and local involvement of the thyroid cancer were associated with higher OM and CSM. All treatment modalities were again associated with lower OM and CSM. Furthermore, age and tumor stage did not interact with each other to affect mortality.

ATC is a rare cancer that tends to occur in older patients compared to differentiated thyroid cancers with a mean age of 65 years old at the time of diagnosis[14,15]. Our study mirrors the literature as most patients were diagnosed between 60 and 79. Previously available literature has highlighted a female predominance with up to 70% in the Kebebew series[14]. A similar trend was seen in the Nagaiah series[15]. Our study results are in adequacy with the current literature with a female involvement close to 60%. More than two thirds of our cohort had distant metastasis at the time of diagnosis. Distant metastasis at the time of diagnosis has been reported in up to 50% of patients in the available literature[16,17].

Most patients with ATC die within a few months, primarily because of local extension and airway obstruction[18]. Thus, tumor size plays an important role in the mortality of patients with ATC even without distant metastasis as shown in the literature[19,20]. A similar trend was observed in our cohort, patients with local invasion had the highest OM and CSM. Distant metastasis has also been shown to be a single predictor of poor outcome in the literature[21,22] with similar results in our cohort. Age has been shown to be an important independent factor of poor prognosis in several rare cancers [23-25]. Although the univariate analysis revealed a poor prognosis associated with advanced age, those results did not hold true in the multivariate analysis while considering covariates. This could be explained by the fact that most patients are diagnosed at advanced age[14,15], an observation that was made in our cohort as well, with most patients diagnosed between 60-79 years old followed by patients 80 years or older.

Several studies addressing cancer mortality have focused on the interaction of two or more independent prognostic factors. Some studies revealed a notable interaction while others did not find any[24,26]. In an effort to better understand factors associated with mortality of this malignancy with a very dismal prognosis, we conducted a study looking at the extent to which two independent prognostic factors would interact to affect mortality. We found that although age and advanced/Locally invasive disease individually affect prognosis, they do not interact to enhance mortality. This novel finding adds to the literature by unveiling an important area of this pathology that has not been studied yet. This finding suggests a novel area of study within ATC prognosis.

It has been demonstrated in the literature that married patients tend to have a lower mortality compared to their non married counterparts[23,26]. It was hypothesized that married patients may have stronger social support leading to a better outcome. However, a recent study on Primary cardiac sarcoma found a lower OM in widowed patients[27]. Interestingly, divorced patients had the best outcome in our cohort. A somehow similar trend was observed in anal canal squamous cell carcinoma where divorced patients had the second-best overall survival following married patients[28]. Our findings can be explained by a lower sample size of divorced patients in our cohort. Furthermore, most patients are diagnosed at an advanced stage and social support may not play a crucial role in mortality as seen in other cancers[23,27].

Although most patients are diagnosed at an advanced stage, all treatment modalities offered were associated with a lower OM and CSM. This finding is extremely important as early detection and management of this dismal cancer may significantly impact mortality. This data may assist treating oncologists in decision making for management of patients with this deadly malignancy. Patients with suspected locally invasive ATC should be promptly evaluated for resection as this may significantly impact their mortality. Historically, metastatic ATC is incurable with a median survival of 4.2 months. However, this data included a period from 1985 and 2009[29]. Our more recent and updated data offers a better prognostic picture. The study suggests a more favorable prognosis for ATC patients compared to historical data, likely due to advancements in targeted therapy and precision oncology. The findings may assist oncologists in decision-making regarding the management of ATC patients, emphasizing the importance of evaluating and managing based on cancer stage rather than age. Furthermore, available treatment modalities should be offered rather than end of life care given the improved mortality associated with our cohort. Overall, the study provides valuable insights into the demographic,

**Table 2 Crude analysis of factors associated with all-cause mortality and cancer related mortality among United States patients diagnosed with anaplastic thyroid cancer between 2010 and 2017**

| Characteristics | Overall mortality | Cancer related mortality |
|---|---|---|
| | Crude proportional, HR (95%CI) | Crude proportional, HR (95%CI) |
| Gender | | |
| Female | 1 (Reference) | 1 (Reference) |
| Male | 0.97 (0.80-1.16) | 0.96 (0.89-1.16) |
| Age at diagnosis, year old | | |
| 00-59 | 1 (Reference) | 1 (Reference) |
| 60-79 | 1.38 (1.10-1.74)[b] | 1.29 (1.02-1.64)[a] |
| 80 + | 1.64 (1.24-2.16)[b] | 1.16 (1.19-2.08)[b] |
| Marital status | | |
| Married | 1 (Reference) | 1 (Reference) |
| Single | 1.00 (0.77-1.30) | 0.94 (0.71-1.23) |
| Divorced/separated | 0.77 (0.54-1.11) | 0.76 (0.52-1.11) |
| Widowed | 1.23 (0.97-1.56) | 1.22 (0.96-1.55) |
| Tumor stage | | |
| Localized | 1 (Reference) | 1 (Reference) |
| Regional by direct extension only | 2.12 (1.22-3.66)[b] | 2.07 (1.16-3.66)[a] |
| Regional lymph nodes involved only | 1.58 (0.81-3.09) | 1.59 (0.79-3.20) |
| Regional by both direct extension and lymph node involvement | 1.75 (1.02-3.00)[a] | 1.82 (1.04-3.19)[a] |
| Distant | 3.24 (2.05-5.12)[b] | 3.25 (2.01-5.24)[b] |
| Race | | |
| Non-Hispanic white | 1 (Reference) | 1 (Reference) |
| Non-Hispanic black | 1.12 (0.79-1.59) | 1.11 (0.78-1.59) |
| Hispanic | 1.23 (0.96-1.58) | 1.22 (0.94-1.57) |
| Other | 1.30 (0.98-1.71) | 1.27 (0.95-1.69) |
| Living area | | |
| Counties in metropolitan areas of 1 million persons | 1 (Reference) | 1 (Reference) |
| Counties in metropolitan areas of 250000 to 1 million persons | 1.11 (0.89-1.38) | 1.11 (0.89-1.39) |
| Counties in metropolitan areas of 250000 persons | 0.74 (0.46-1.18) | 0.77 (0.47-1.24) |
| Nonmetropolitan counties adjacent to a metropolitan area | 0.80 (0.55-1.14) | 0.80 (0.55-1.16) |
| Nonmetropolitan counties not adjacent to a metropolitan area | 1.41 (0.93-2.15) | 1.49 (0.98-2.27) |
| Income per year | | |
| $ < $45000 | 1 (Reference) | 1 (Reference) |
| $45000-54999 | 0.92 (0.55-1.54) | 0.86 (0.51-1.45) |
| $55000-64999 | 1.35 (0.85-2.17) | 1.28 (0.80-2.06) |
| $65000-74999 | 1.01 (0.64-1.60) | 0.96 (0.60-1.52) |
| $75000 + | 0.90 (0.57-1.43) | 0.85 (0.54-1.34) |
| Radiation | | |
| No | 1 (Reference) | 1 (Reference) |
| Yes | 0.61 (0.50-0.74)[b] | 0.59 (0.49-0.72)[b] |

| | | |
|---|---|---|
| Chemotherapy | | |
| No | 1 (Reference) | 1 (Reference) |
| Yes | 0.61(0.50-0.73)[b] | 0.60(0.49-0.72)[b] |
| Surgery | | |
| No | 1 (Reference) | 1 (Reference) |
| Yes | 0.50 (0.41-0.59)[b] | 0.47 (0.39-0.58)[b] |

[a]$P < 0.05$.
[b]$P < 0.01$.
HR: Hazard ratio.

**Table 3 Multivariate cox proportional hazard regression analyses of factors affecting all-cause mortality and cancer related mortality among United States patients diagnosed with anaplastic thyroid cancer between 2010 and 2017**

| Characteristics | Overall mortality | Cancer related mortality |
|---|---|---|
| | Adjusted proportional, HR (95%CI) | Adjusted proportional, HR (95%CI) |
| Gender | | |
| Female | 1 (Reference) | 1 (Reference) |
| Male | 1.11 (0.89-1.38) | 1.09 (0.87-1.36) |
| Age at diagnosis, year old | | |
| 00-59 | 1 (Reference) | 1 (Reference) |
| 60-79 | 2.85 (0.61-13.43) | 2.16 (0.46-10.18) |
| 80 + | 2.01 (0.40-10.18) | 1.48 (0.29-7.60) |
| Marital status | | |
| Married | 1 (Reference) | 1 (Reference) |
| Single | 1.00 (0.75-1.34) | 0.94 (0.69-1.26) |
| Divorced/separated | 0.63 (0.42-0.94)[a] | 0.61 (0.40-0.92)[a] |
| Widowed | 1.03 (0.78-1.37) | 1.03 (0.77-1.37) |
| Tumor stage | | |
| Localized | 1 (Reference) | 1 (Reference) |
| Regional by direct extension only | 6.26 (1.29-30.42)[a] | 5.05 (1.05-24.19)[a] |
| Regional lymph nodes involved only | 2.71 (0.42-17.49) | 2.30 (0.36-14.68) |
| Regional by both direct extension and lymph node involvement | 3.58 (0.74-17.31) | 2.97 (0.62-14.15) |
| Distant | 5.73 (1.34-24.51)[a] | 4.57 (1.08-19.29)[a] |
| Race | | |
| Non-Hispanic white | 1 (Reference) | 1 (Reference) |
| Non-Hispanic black | 0.91 (0.62-1.35) | 0.90 (0.60-1.34) |
| Hispanic | 1.16 (0.90-1.51) | 1.13 (0.87-1.49) |
| Other | 1.14 (0.85-1.54) | 1.12 (0.83-1.52) |
| Living area | | |
| Counties in metropolitan areas of 1 million persons | 1 (Reference) | 1 (Reference) |
| Counties in metropolitan areas of 250000 to 1 million persons | 1.26 (0.99-1.60) | 1.26 (0.99-1.61) |
| Counties in metropolitan areas of 250000 persons | 0.76 (0.46-1.27) | 0.82 (0.49-1.38) |

| | | |
|---|---|---|
| Nonmetropolitan counties adjacent to a metropolitan area | 0.79 (0.50-1.26) | 0.80 (0.50-1.29) |
| Nonmetropolitan counties not adjacent to a metropolitan area | 1.19 (0.70-2.01) | 1.25 (0.74-2.13) |
| Income per year | | |
| $ < $45000 | 1 (Reference) | 1 (Reference) |
| $45000-54999 | 0.75 (0.43-1.31) | 0.72 (0.41-1.26) |
| $55000-64999 | 1.04 (0.59-1.82) | 1.01 (0.57-1.77) |
| $65000-74999 | 0.85 (0.48-1.51) | 0.84 (0.47-1.50) |
| $75000 + | 0.74 (0.42-1.33) | 0.71 (0.40-1.29) |
| Radiation | | |
| No | 1 (Reference) | 1 (Reference) |
| Yes | 0.66 (0.53-0.83)[b] | 0.64 (0.51-0.81)[b] |
| Chemotherapy | | |
| No | 1 (Reference) | 1 (Reference) |
| Yes | 0.63 (0.50-0.79)[b] | 0.62 (0.50-0.78)[b] |
| Surgery | | |
| No | 1 (Reference) | 1 (Reference) |
| Yes | 0.53 (0.43-0.66)[b] | 0.51 (0.41-0.63)[b] |

[a]$P < 0.05$.
[b]$P < 0.01$.
HR: Hazard ratio.

**Table 4 Multivariate cox proportional hazard regression analyses of factors affecting all-cause mortality and anaplastic thyroid cancer related mortality among United States patients between 2010 and 2017 taking into account the interaction between tumor stage and age**

| Tumor stage and age (stage & age) | OM | CSM |
|---|---|---|
| I & 00-59 | 1 | 1 |
| II & 00-59 | 1 | 1 |
| II & 60-79 | 0.28 (0.06-1.62) | 0.31 (0.05-1.85) |
| II & 80 + | 0.32 (0.05-2.08) | 0.43 (0.06-2.79) |
| III & 00-59 | 1 | 1 |
| III & 60-79 | 0.43 (0.05-3.63) | 0.44 (0.05-3.89) |
| III & 80 + | 0.70 (0.08-6.13) | 0.91 (0.10-8.07) |
| IV & 00-59 | 1 | 1 |
| IV & 60-79 | 0.53 (0.09-2.99) | 0.65 (0.11-3.65) |
| IV & 80 + | 0.52 (0.07-3.90) | 0.70 (0.09-5.29) |
| V & 00-59 | 1 | 1 |
| V & 60-79 | 0.44 (0.09-2.09) | 0.54 (0.11-2.58) |
| V & 80 + | 0.70 (0.13-3.66) | 0.87 (0.16-4.63) |

CSM: Cancer specific mortality; OM: Overall mortality.

clinical, and prognostic factors of ATC, highlighting the importance of early detection, prompt management, and the potential impact of treatment modalities on patient outcomes.

Certain limitations must be considered in the interpretation of the results of this study. The information available for chemotherapy did not specify if this treatment was given as neoadjuvant or adjuvant. Furthermore, the SEER database, the largest cancer database in the United States that is publicly available, does not provide information on comorbidities.

## CONCLUSION

In conclusion, our ATC mortality study from 2010 to 2017 reveals notable trends: Heightened risk in the 80 + age group, with distant metastasis and residing in nonmetropolitan areas without adjacency to a metropolitan center. A distinctive finding is the protective effect among divorced/separated individuals. This aligns with existing literature, emphasizing age and tumor stage as crucial influencers in ATC outcomes. From a clinical perspective, recognizing the vulnerability of the elderly and the impact of marital status informs targeted interventions. Regional disparities in nonmetropolitan areas highlight the need for focused healthcare strategies. Future research should probe mechanisms behind the protective effect among divorced/separated individuals and address healthcare disparities in nonmetropolitan areas. Our study provides a foundation for understanding ATC mortality factors, guiding the development of personalized healthcare strategies.

## FOOTNOTES

**Author contributions:** Bangolo A and Fwelo P designed the research study; Bangolo A, Auda A, Bukasa-Kakamba J, Bhakta N, Dey S, Lilhori A, Reddy G, Alqinai B, Sidiqui A, Sekhon I, Khatiashvili B, Abbas I, Kunnel S, Jarri A, Martinez E, Daoud D, Gupta I, Gompa H, Pender S, Aljaberi D performed the research; Bangolo A, Aljanaahi H, Kunnel SS, Xiao Y, Jung Y, Nagpaul S, Naz A, Mallela T, Maung PM, Khalaf IY, Kim S, Alrestom R, Gajera A, Alkealy H, Kansal D, Dhall S, Satheesha S, Weissman S analyzed the data and wrote the manuscript; All authors have read and approved the final manuscript.

**Conflict-of-interest statement:** No potential conflict of interest was reported by the authors.



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**S-Editor:** Liu JH
**L-Editor:** A
**P-Editor:** Wang WB

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World Journal of Experimental Medicine

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*World J Exp Med* 2024 September 20; 14(3): 92589

DOI: 10.5493/wjem.v14.i3.92589

ISSN 2220-315x (online)

ORIGINAL ARTICLE

Basic Study

# Amylase intrapancreatic infusion delays insulin release during an intravenous glucose tolerance test, proof of acini–islet–acinar interactions

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**Specialty type:** Medicine, research and experimental

**Provenance and peer review:** Unsolicited article; Externally peer reviewed.

**Peer-review model:** Single blind

**Peer-review report's classification**

**Scientific Quality:** Grade C, Grade C

**Novelty:** Grade B, Grade C

**Creativity or Innovation:** Grade B, Grade C

**Scientific Significance:** Grade B, Grade C

**P-Reviewer:** Srinivasan AR

**Received:** January 31, 2024

**Revised:** June 19, 2024

**Accepted:** July 10, 2024

**Published online:** September 20, 2024

**Processing time:** 210 Days and 14.2 Hours

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## Abstract

***BACKGROUND***

The possible existence of an acini–islet–acinar (AIA) reflex, involving mutual amylase and insulin interactions, was investigated in the current acute experiment on pigs.

***AIM***

To confirm the existence of an AIA reflex and justify the placement of the exocrine and endocrine pancreatic components within the same organ.

***METHODS***

The study was performed on six pigs under general anesthesia. An intravenous glucose tolerance test was performed, with a bolus infusion of 50% glucose to the jugular vein, while amylase (5000 U/kg) or vehicle intrapancreatic infusions were administered *via* the pancreaticoduodenalis cranialis artery during 30 min with a 1 mL/min flow rate.

***RESULTS***

The amylase infusion to pancreatic arterial circulation inhibited and delayed the insulin release peak which is usually associated with the highest value of blood glucose and is typically observed at 15 min after glucose infusion, for > 1 h. The intrapancreatic infusion of the vehicle (saline) did not have any effect on the time frame of insulin release. Infusion of 1% bovine serum albumin changed the insulin release curve dramatically and prolonged the high range of insulin secretion, far beyond the glucose peak.

***CONCLUSION***

Intrapancreatic arterial infusion of amylase interrupted the integrated glucose–insulin interactions. This confirms an AIA reflex and justifies placement of the exocrine and endocrine pancreatic components within the same organ.


**Key Words:** Amylase; Glucose-insulin-amylase interaction; Intravenous glucose tolerance test; Acini-islet-acinar axis; Insulin



**Core tip:** The acini–islet–acinar axis involves the inhibition of insulin production by amylase and the stimulation of amylase release by insulin, which is of particular importance with regards to glucose regulation. There is increasing evidence that amylase levels correspond to overall metabolic health, with low amylase levels being significantly associated with the development of metabolic syndrome, diabetes, and obesity. Disruption of the integrated glucose–insulin interactions by intrapancreatic arterial amylase infusion in the present study confirms the existence of an AIA reflex and justifies placement of the exocrine and endocrine pancreatic components within the same organ.

**Citation:** Pierzynowska K, Wychowański P, Zaworski K, Woliński J, Donaldson J, Szkopek D, Roszkowicz-Ostrowska K, Kondej A, Pierzynowski SG. Amylase intrapancreatic infusion delays insulin release during an intravenous glucose tolerance test, proof of acini–islet–acinar interactions. *World J Exp Med* 2024; 14(3): 92589

**URL:** https://www.wjgnet.com/2220-315x/full/v14/i3/92589.htm

**DOI:** https://dx.doi.org/10.5493/wjem.v14.i3.92589

## INTRODUCTION

The postulated acini–islet–acinar (AIA) reflex, which integrates and justifies the existence of the exocrine and endocrine pancreas within the same organ, requires the presence of amylase in the interstitial fluid surrounding the pancreatic islets and acinar cells[1].

Another contemporary question in terms of gastroenterology is how the pancreatic enzymes reach the bloodstream and whether such a transition is a sign of health or disease. For example, pancreatitis development was believed to be associated with high serum amylase levels at the beginning of the century[2]. Nowadays, this hypothesis has been abandoned and high serum amylase levels are recognized to be noninformative for the diagnosis of pancreatitis. There is a growing number of publications to date that show a correlation between serum amylase levels and metabolic health, with low amylase levels being significantly associated with the development of metabolic syndrome, diabetes and obesity [3,4].

Leakage of the pancreatic enzymes from the acinar cells to the interstitial fluid is the commonly accepted hypothesis regarding the source of pancreatic enzymes in the bloodstream. The approximate concentration of pancreatic enzymes in the interstitial fluid that would ensure their existing levels in the peripheral blood (often measured for diagnostic purposes), needs to be several fold or even several hundred fold higher than their concentration observed in the blood.

Amylase, infused *via* the pancreaticoduodenalis cranialis artery into the pancreas should penetrate the endothelium in the pancreatic vascular system, and its concentration in the infusate should be high, to influence amylase levels in the interstitial space. Since amylase and other enzymes are smaller than the estimated size of the endothelial holes of 70 kD, which block larger macromolecules (*e.g.*, albumins) from migration to the intestinal fluid, exogenous amylase can enter the pancreatic interstitial fluid.

The aim of the present study was to prove that exogenously increased levels of amylase in the pancreatic interstitial fluid would inhibit insulin release.

## MATERIALS AND METHODS

### Animals

The acute experiment was performed on six, Swedish Landrace × Yorkshire × Hampshire pigs of both genders (40–50 kg), that were fasted overnight. The pigs were premedicated intramuscularly, for sedation, with a mixture of: medetomidine 1 mg/m$^2$, midazolam 5 mg/m$^2$, ketamine 8 mg/kg and methadone 0.2 mg/kg. An intravenous cannula was inserted into the marginal ear vein. Drugs to induce anesthesia were administered through the cannula: propofol 2 mg/kg and lidocaine 1 mg/kg. Preoxygenation of 3 L/min with a mask was started during induction of anesthesia. The incision site was epidurally anesthetized with 1% lidocaine. Anesthesia was maintained with isoflurane a concentration of 1%–1.5% and oxygen flow at 1.5–2.0 L/min. An analgesic precaution of intramuscular metamizole at 50 mg/kg and intravenous bolus of fentanyl at 2 μg/kg was administered, followed by a constant-rate infusion of fentanyl in 0.9% NaCl throughout the operation, at a dose of 1.5 μg/kg/h. Monitoring of vital functions [blood oxygen saturation (spO2), end tidal carbon dioxide ($EtCO_2$), pulse rate (PR), noninvasive blood pressure (NIBP): systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), temperature] was performed throughout the operation. The pigs were placed on a surgical heated table, in a dorsal position and were under general anesthesia during the entire experiment, which was performed at the Large Animal Models Laboratory, The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jabłonna, Poland. The study was approved by the Second Local Ethics Committee for Animal Experimentation in Warsaw, Poland (approval No. WAW2/025/2022).

### Catheter implantation surgery

After the surgical level of anesthesia was reached, jugular vein catheters were surgically implanted in the pigs. Following the jugular vein catheter surgery, the abdominal cavity of each pig was opened, along the linea alba, directly after the sternum, with a 15-cm long incision. After localization of the gastroduodenalis artery, the bifurcation of the pancreaticoduodenalis cranialis artery was located (Figure 1). From this point onwards the gastroduodenalis artery becomes the right gastric epiploic artery. The latter was then gently dissected, 2–3 cm cranially to the pancreaticoduodenalis cranialis artery. Two situation silk 3-0 sutures were placed under the right gastric epiploic artery in the place of its dissection and an incision was made with microtip scissors.

A silastic catheter, with an outer diameter of 0.02 mm, was inserted through the incision in the epiploic artery, towards the pancreaticoduodenalis cranialis artery. The tip of the catheter was situated 2–3 cm behind the pancreaticodoudenalis cranialis artery bifurcation from the gastroduodenalis artery and the right epiploic artery was sutured shut, under and over the inserted catheter. Blood flow to the pancreas and duodenum was ensured *via* the gastroduodenalis artery, while blood flow to the right epiploic artery was supplied *via* the left epiploic artery. All infusions to the catheter with the tip beyond the pancreaticodoudenalis cranialis artery were directed to the pancreas and duodenum. Approximately 70% of the blood supply to the pancreas originates from the pancreaticoduodenalis cranialis artery in pigs. Localization of the catheter tips and confirmation of the infusion to the pancreas was confirmed using Evans blue, postmortem.

### Experimental design

After insertion of the catheters into the jugular vein and gastroduodenalis artery, the pigs were left to stabilize for 30 min. Anesthesia was induced and maintained almost identical for all pigs for the duration of the experiment, with necessary adjustments to ensure animal wellbeing. After the 30-min stabilization period, a baseline blood sample was obtained and the respective infusions begun. A bolus injection of 50% glucose (1 g/kg) to the jugular vein catheter, lasting for 2 min, with a parallel 30-min infusion of (with an infusion rate of 1 mL/min into the pancreatic arterial circulation) either: (1) α-amylase (5000 U/kg) in saline – pigs 1 and 4; (2) 1%, bovine serum albumin (BSA) in saline – pigs 2 and 3; or (3) saline (0.9 % NaCl) – pigs 5 and 6. After the 2-h experiment, the pigs were killed by overdose of pentobarbital, infused *via* the jugular vein catheter.

### Blood sampling

Blood samples obtained during the experiment were collected *via* the jugular vein catheter at 1 min prior to glucose infusion and at 15, 30, 60, 90 and 120 min after the glucose infusion and transferred to BD Vacutainer® glass $K_3$EDTAtubes (BD Diagnostics, Franklin Lakes, NJ, USA).

The blood samples were immediately placed on ice before they were centrifuged at 3000 × g for 15 min at 4°C, and plasma was separated and stored at -80°C until further analysis. Blood glucose concentrations were measured directly following blood sampling using a glucometer and test strips (Accu-Chek Aviva, Roche Diagnostics, Germany). Plasma insulin concentrations were measured using a porcine insulin ELISA kit (cat# 10-1200-01; Mercodia, Uppsala, Sweden).

### *Statistical analysis*

Data are expressed as individual values. Area under the curve (AUC) values are expressed as mean ± SD. AUC calculations were carried out using Prism, version 10 (GraphPad Software, San Diego, CA, USA).

## RESULTS

Before the main experiment was performed, several pilot studies were done to investigate the impact of the anesthesia and manipulation with arterial catheter implantation on the insulin response during the intravenous glucose tolerance test (IVGTT).

All parameters that were monitored while the pigs were under anesthesia, such as spO2 (97%–100%), $EtCO_2$ (35–45 mmHg), PR (70–100 bpm), SBP (120–140 mmHg), DBP (60–80 mmHg), MAP (60–100 mmHg), and temperature (37.5–38.5°C) were maintained within the physiological range during the entire experimental period and anesthesia. Based on the above-mentioned parameters on the cardiac monitor, the anesthesiologist regulated the depth of anesthesia by altering the concentration of isoflurane and oxygen flow in the respiratory circuit or administered propofol through a venflon in the marginal ear vein.

In pigs infused with amylase to the intrapancreatic arterial circulation (pigs 1 and 4), glucose levels during the IVGTT reached a peak, increasing from 80–100 mg/dL to 450–500 mg/dL, at 15 min after glucose injection (Figure 2A).

Surprisingly, peak blood insulin levels did not match peak glucose levels and were observed as late as 90 min after glucose injection and persisted until the last measured point at 2 h following glucose injection. Peak insulin levels varied between pigs, ranging from 5 to 23 mU/L, while basal levels were around 1 mU/L (Figure 2B), and were still on the same level as that measured at the 15-min time point, when the highest blood glucose values were observed.

In pigs that obtained an intrapancreatic infusion of 0.9% NaCl and 1% BSA, blood glucose levels were similar to those observed in pigs treated with amylase and reached a peak at 15 min following glucose infusion, from 60–100 to 450–470 mg/dL (Figures 3A and 4A, respectively).

Insulin levels peaked at 15 min after glucose infusion from 2 to 10–20 mU/L in pigs that obtained an intrapancreatic infusion of 0.9% NaCl as a vehicle (pigs 5 and 6). Insulin levels in these pigs returned to basal values at 30 min after glucose infusion. In pigs treated with 1% BSA (pigs 2 and 3), insulin levels increased at 15 min after glucose infusion, to 15–29 mU/L, and then reached a plateau of 22–30 mU/L and remained stable during all measurements at 30, 60, 90 and 120 min following glucose infusion (Figures 3B and 4B).

## DISCUSSION

During the pilot experiments, the effects of the anesthesia on the insulin response during an IVGTT was evaluated, since previous studies showed that isoflurane anesthesia reduces the reactivity of insulin to glucose during infusion[5]. The results of the pilot studies, as well as the latter experimental results from a previous study confirm that isoflurane could reduce the insulin response to an IVGTT and limit glucose metabolism, as compared to the results obtained from conscious pigs[6]. However, in our experiment on conscious, healthy pigs, we did not observe a shift in the insulin response or a prolonged insulin response during the IVGTT. In contrast, a slight delay and long-lasting insulin response to the IVGTT was observed in exocrine-pancreas-insufficient pigs in a previous study[7]. Following the pancreatic duct ligation (PDL) surgery, the amylase concentration in the pancreatic interstitial fluid is increased. In that way our infusion of amylase to the intrapancreatic arterial circulation can mimic PDL-provoked changes in amylase concentration in the interstitial fluid. A previous study by our laboratory showed a shift in the insulin response during an intraduodenal glucose tolerance test in low dose streptozotocin treated pigs which develop type 2 diabetes, following parallel amylase infusion[6].

The current pancreatic perfusion experiment was performed to prove the existence of an AIA axis, in that the infusion of exogenous amylase, *via* the arterial circulation to the pancreas, could to some extent mimic the appearance of host amylase in the interstitial fluid, as well as its influence on the insulin-producing cells in the pancreatic islets of Langerhans. The acute porcine model for local infusion of agents, exclusively to the pancreas or duodenum, which has previously been used in our laboratory[8,9], was employed in the present study for the local arterial intrapancreatic infusions (Figure 1).

Both α-amylase, with a molecular weight of 58.4 kDa, as well as BSA, with molecular weight of 66 kDa, can partially (not easily) penetrate the interstitial fluid when applied arterially, since it is postulated that the endothelium of blood vessels is permeable to molecules < 70 kDa. Thus, it was assumed that amylase as the test molecule and BSA, which was used as a positive control, as well as 0.9% NaCl which was used as a vehicle, could reach the interstitial fluid and finally, the beta cells in the pancreatic islets. Considering the postulation by Lifson *et al*[10], concerning the existence of blood vessel circuits in the pancreas, similar to the renal and liver rete mirabille arterio arteriosum and vene venosum, one could suspect that infused molecules would reach both the islets of Langerhans and the acinar cell structures. The specificity of the pancreatic islets blood flow[11] additionally ensures that amylase infused to the pancreatic arterial circulation can reach the endocrine pancreas.

Amylase infusion in the current study delayed the glucose-stimulated insulin release for > 1 h during the IVGTT. There was no enhancing of insulin release even despite the parallel loading of glucose, with blood glucose levels reaching an extraordinarily high peak (400 mg/dL) within 15 min of loading. Importantly the inhibitory effect of amylase on insulin

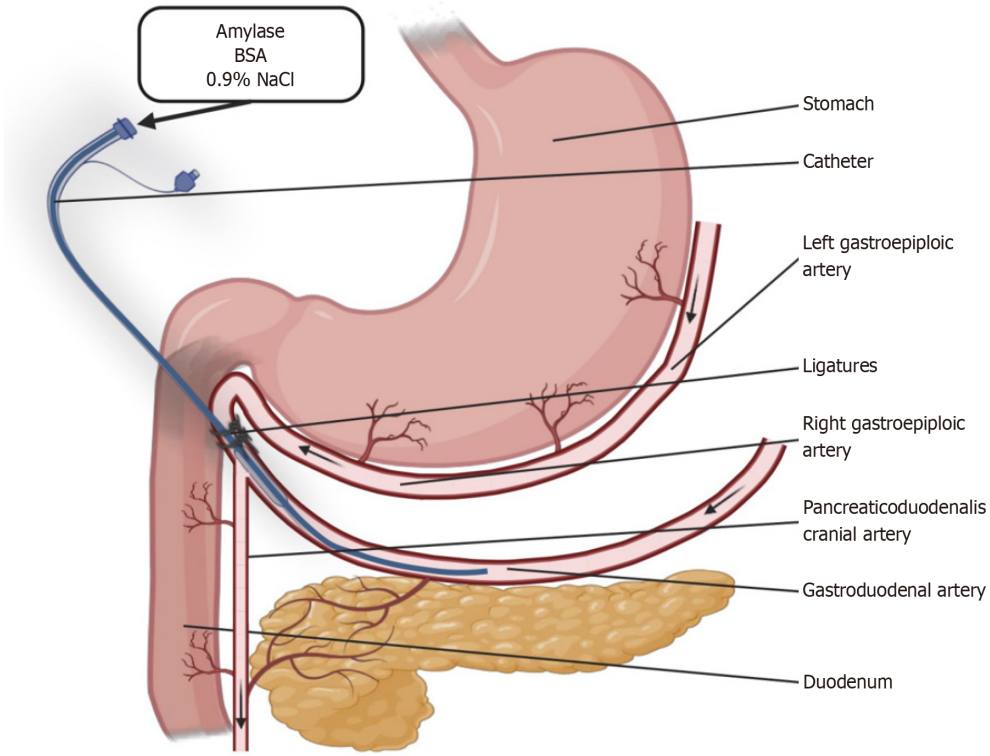


**Figure 1 Schematic diagram showing the placement of the catheter, ensuring infusions exclusively to the pancreas and partially to the duodenum.** BSA: Bovine serum albumin.

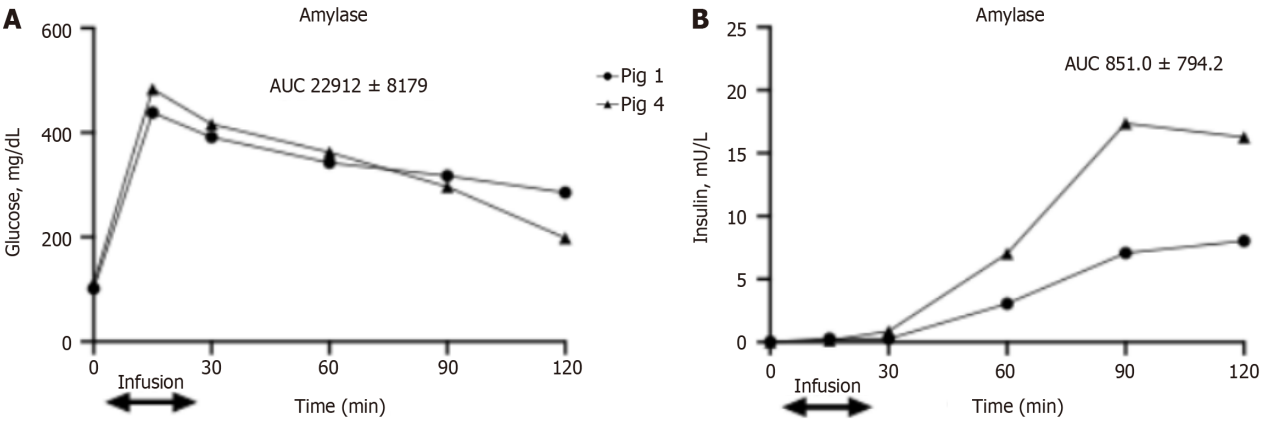


**Figure 2 Glucose and insulin levels after the intravenous glucose tolerance test in pigs 1 and 4 during infusion of amylase to the intrapancreatic arterial circulation.** A: Blood glucose levels; B: Plasma insulin levels. Data are expressed as individual values, data on area under the curve are expressed as mean ± SD. AUC: Area under the curve.

release was still observed for a further 30 min after the amylase infusion was stopped. Neither the NaCl infusion nor the 1% BSA infusion inhibited the immediate insulin release observed in response to the glucose loading.

In contrast, BSA infusion to the pancreatic artery strongly enhanced insulin secretion for the entire experimental period of 2 h. This observation needs to be further investigated in future studies to highlight the effects of plasma proteins on insulin release and resistance[12].

## CONCLUSION

In summary, the inhibition of insulin secretion by intrapancreatic amylase confirms the existence of an AIA reflex. The results of the present study should however be considered in the context of a serious limitation in that acute conditions can affect the sensitivity of insulin release during an IVGTT[13]. Thus, similarly designed, chronic experiments are going to be carried out in the near future.

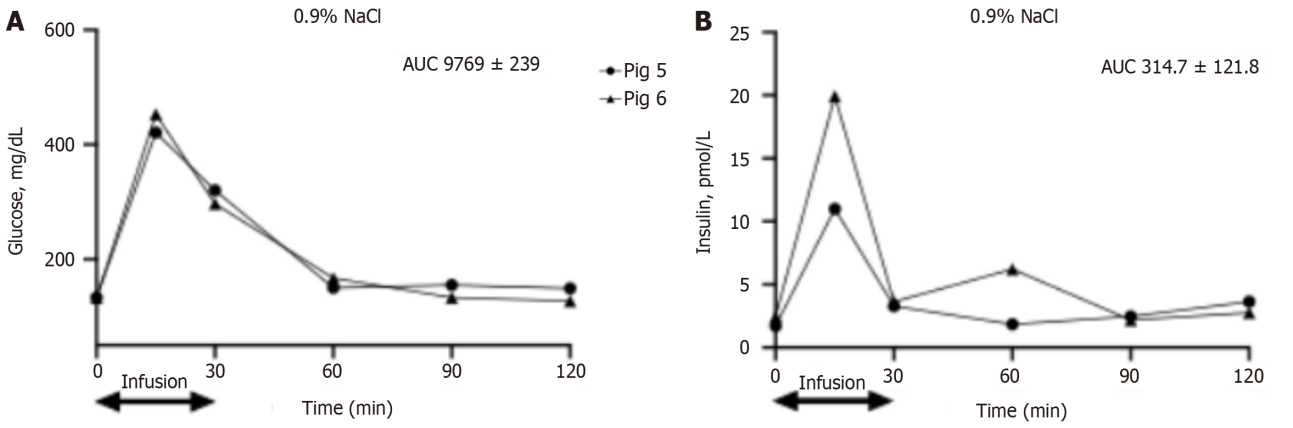


**Figure 3 Glucose and insulin levels after the intravenous glucose tolerance test in pigs 5 and 6 during infusion of amylase to the intrapancreatic arterial circulation.** A: Blood glucose levels; B: Plasma insulin levels. Data are expressed as individual values, data on area under the curve are expressed as mean ± SD. AUC: Area under the curve.

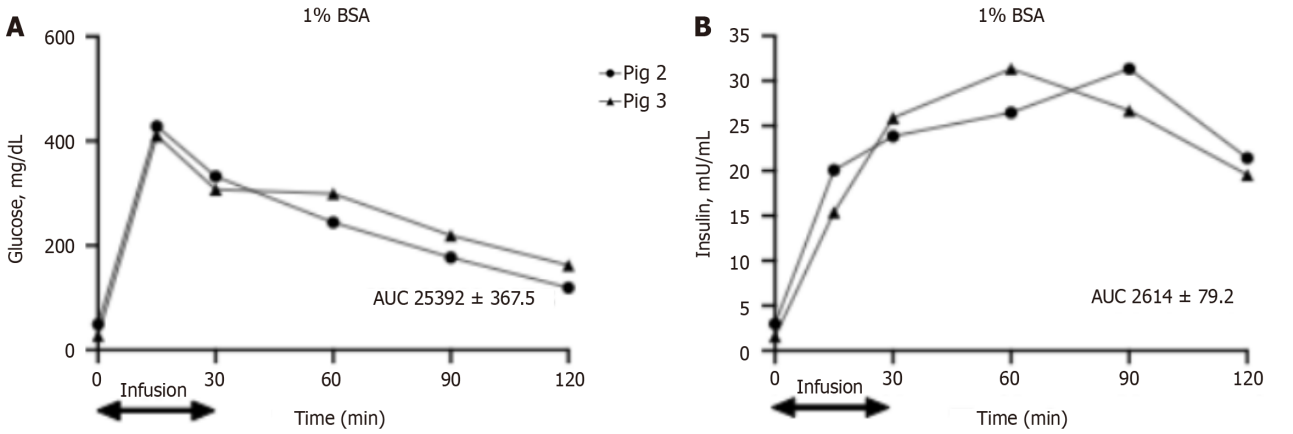


**Figure 4 Glucose and insulin levels after the intravenous glucose tolerance test in pigs 2 and 3 during infusion of amylase to the intrapancreatic arterial circulation.** A: Blood glucose levels; B: Plasma insulin levels. Data are expressed as individual values, data on area under the curve are expressed as mean ± SD. AUC: Area under the curve; BSA: Bovine serum albumin.

## FOOTNOTES

**Author contributions:** Wychowanski P, Pierzynowska K, and Pierzynowski S for conceptualization; Pierzynowska K and Zaworski K for data curation, formal analysis; Wychowanski P, Pierzynowski SG, and Pierzynowska K for funding acquisition; Pierzynowska K, Wychowanski P, Zaworski K, Woliński J, Szkopek D, Roszkowicz K, Kondej A, and Pierzynowski S for investigation; Pierzynowska K, Zaworski K and Wychowanski P for methodology; Woliński J for project administration; Donaldson J, Roszkowicz-Ostrowska K for resource.

**Institutional review board statement:** The National Science Centre (Poland) has approved the protocol of current study.

**Institutional animal care and use committee statement:** The animal study protocol was approved by the Second Local Ethics Committee for Animal Experimentation in Warsaw, Poland (approval No. WAW2/025/2022). The protocol was designed to minimize pain or discomfort to the animals.

**Conflict-of-interest statement:** The authors of this manuscript have the following competing interests: Stefan Pierzynowski and Kateryna Pierzynowska are the owners of Anara AB, Sweden. Piotr Wychowański is the owner of MED Sp. z o.o., Poland. Stefan Pierzynowski is the owner of Vitanano Sp. z o.o., Poland. Kamil Zaworski received funding from National Science Centre, Poland (Preludium, 2021/41/N/NZ9/01722). All other authors declare no competing interests.

**Data sharing statement:** Data is contained within the article. No additional data are available.

**ARRIVE guidelines statement:** The authors have read the ARRIVE guidelines, and the manuscript was prepared and revised according to the ARRIVE guidelines.

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**S-Editor:** Liu H
**L-Editor:** Kerr C
**P-Editor:** Wang WB

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World Journal of Experimental Medicine

Submit a Manuscript: https://www.f6publishing.com

*World J Exp Med* 2024 September 20; 14(3): 95565

DOI: 10.5493/wjem.v14.i3.95565

ISSN 2220-315x (online)

*SYSTEMATIC REVIEWS*

# Addressing trauma, post-traumatic stress disorder, and post-traumatic growth in breast cancer patients

Amos Nnaemeka Amedu

**Specialty type:** Medicine, research and experimental

**Provenance and peer review:** Invited article; Externally peer reviewed.

**Peer-review model:** Single blind

**Peer-review report's classification**

**Scientific Quality:** Grade B

**Novelty:** Grade A

**Creativity or Innovation:** Grade B

**Scientific Significance:** Grade B

**P-Reviewer:** Ghimire R, Nepal

**Received:** April 12, 2024

**Revised:** May 14, 2024

**Accepted:** June 11, 2024

**Published online:** September 20, 2024

**Processing time:** 138 Days and 12.5 Hours

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## Abstract

*BACKGROUND*

Breast cancer (BC) is a common cancer among females in Africa. Being infected with BC in Africa seems like a life sentence and brings devastating experiences to patients and households. As a result, BC is comorbid with trauma, post-traumatic stress disorder (PTSD), and post-traumatic growth (PTG).

*AIM*

To identify empirical evidence from peer-reviewed articles on the comorbidity trajectories between BC and trauma, BC and PTSD, and BC and PTG.

*METHODS*

This review adhered to the PRISMA guidelines of conducting a systematic review. Literature searches of the National Library of Medicine, Scopus, PubMed, Google Scholar, and Scopus databases were conducted using search terms developed for the study. The search hint yielded 769 results, which were screened based on inclusion and exclusion criteria. At the end of the screening, 24 articles were included in the systematic review.

*RESULTS*

BC patients suffered trauma and PTSD during the diagnosis and treatment stages. These traumatic events include painful experiences during and after diagnosis, psychological distress, depression, and cultural stigma against BC patients. PTSD occurrence among BC patients varies across African countries, as this review disclosed: 90% was reported in Kenya, 80% was reported in Zimbabwe, and 46% was reported in Nigeria. The severity of PTSD among BC patients in Africa was based on the test results communicated to the patients. Furthermore, this review revealed that BC patients experience PTG, which involves losing, regaining, and surrendering final control over the body, rebuilding a personified identity, and newfound appreciation for the body.

*CONCLUSION*
Patients with BC undergo numerous traumatic experiences during their diagnosis and treatment. Psychological interventions are needed in SSA to mitigate trauma and PTSD, as well as promote PTG.

**Key Words:** Trauma; Post-traumatic stress disorder; Post-traumatic growth; Breast cancer; Patients; Sub-Saharan Africa



**Core Tip:** A high incidence of breast cancer (BC) is common among African females, and a diagnosis thereof is misconstrued as a death sentence because of the low survival rate. BC affects female patients from 25 years to 65 years of age and it is associated with psychological problems such as trauma and post-traumatic stress disorder (PTSD). However, there is a lack of pooled empirical evidence on the comorbidity of BC, trauma, PTSD, and post-traumatic growth among female African patients.

**Citation:** Amedu AN. Addressing trauma, post-traumatic stress disorder, and post-traumatic growth in breast cancer patients. *World J Exp Med* 2024; 14(3): 95565
**URL:** https://www.wjgnet.com/2220-315x/full/v14/i3/95565.htm
**DOI:** https://dx.doi.org/10.5493/wjem.v14.i3.95565

## INTRODUCTION

Breast cancer (BC) is a global disease[1]. A report from the Global Cancer Observatory revealed that BC has an incidence of 30.3% in female patients with cancer, irrespective of age, and caused approximately 685000 deaths in 2020[2,3], while in 2022, 2.3 million cases were reported and 670000 deaths were recorded globally[4]. The American Cancer Society estimated that, in 2024, 310720 new cases of invasive BC will be diagnosed, as well as 56500 new cases of ductal carcinoma *in situ*, while approximately 42250 women will die from BC[5,6]. BC is also the most common type of cancer diagnosed among women in sub-Saharan African countries (SSA)[7,8]. SSA countries have the maximum age-standardized prevalence rate of 17.3 per 100000 women per year. The North African region has the highest occurrence of BC patients, with an age-standardized incidence of 43.2 per 100000 women per year[9] followed by Africa and Southern Africa countries with age-standardized occurrences of 38.6 and 38.9 per 100000 women per year, respectively[8].

BC occurs in every country worldwide. Furthermore, BC affects women, irrespective of age after puberty. However, there is a low prevalence of BC and a low death rate in countries with a very high human development index (HDI), whereas in countries with a low HDI, there is a significant prevalence of BC and high associated death rates[10]. In some parts of SSA, there is a misconception that BC cannot be cured[2]. Hence, BC ailment triggers psychological problems such as trauma and post-traumatic stress disorder (PTSD), and the recovery process known as post-traumatic growth (PTG) immediately after treatment starts.

Trauma is one of the psychological problems that has a severe emotional and biological stress reaction to an unusual event experienced as potentially harmful or aversive[11]. It has been conceptualized as the occurrence of events, victim experiences of the events, and the extent of emotional, physical, and social impacts[12].

BC diagnosis is a sudden traumatic event associated with doubt about the future and changes in social responsibility and relationships[13,14]. BC has been identified as a threat to patients' identities, cultural ideals of feminism, beauty, sexuality, and maternal potential[15]. Individuals with BC habitually experience additional psychological difficulties due to the changes they undergo in their body, as well as family, social, and career roles. These unpleasant BC experiences usually result in traumatic experiences for the patient[16]. Women who have experienced trauma before reported severe BC-related traumatic symptoms and that childhood emotional abuse substantially predicted BC-related intrusive symptoms independently[17]. This report suggests that BC diagnosis has the highest chances of triggering cognitive and emotional reactions that are associated with patients' previous trauma experiences.

Patients may experience BC and PTSD as comorbid illnesses. PTSD is a flashback of traumatic experiences emanating from patients' severe experiences of traumatic events[18]. Cancer has been recognized by the American Psychiatric Association as a traumatic stressor that could precipitate PTSD[19]. PTSD has three diagnostic characteristics, which include re-experiencing, avoidance, negative cognitions and moods, and arousal[20]. Prevalence of PTSD among BC patients is between 3%-32%[21]. Further, there are several factors that contribute to the occurrence of PTSD in BC patients, including the stage of cancer, prognosis, treatment method, extent of pain, social support, hospitalization, and the educational level of the patient[22,23].

In addition, despite the negative feelings BC patients experience after cancer diagnosis, they also experience positive effects, like realizing internal growth, shifting perceptions about life, and establishing rich interpersonal relations, which are all attributes of PTG[14]. PTG is designed to promote psychological adaptation, quality of life, facilitate health promotion behavior, and increase resistance and survival rates among BC patients[14,23]. PTG has three components, which include the perception of change in self (personal empowerment and resilience), change in feeling toward others (accepting various attitudes, improved compassion, and sympathy), and change in life philosophy (changing to feel out

of control, helpless, and alienated)[24].

This review aimed to scope the current literature to map out empirical evidence from peer review articles on the trajectory of comorbidity between BC and trauma, BC and PTSD, and BC and PTG. Existing literature reviews, which examined the relationships between BC and trauma, BC and PTSD[25], and BC and PTG[26], were limited in scope because they examined the linear connection between the two variables in isolation, which is only a fraction of the current review. Nevertheless, the purpose of this review was to provide an updated empirical comparison of BC patient experiences with traumatic events, PTSD, and PTG, as well as their comorbidities. Based on extensive research of online databases, there was no existing literature in this regard; hence, this review is the first of its kind to holistically review the pooled trajectory of BC patient experiences of trauma to the recovery stage. This review may provide substance to mental health practitioners and cancer patients because it reveals verified and updated experiences of BC patients in relation to the concepts of trauma and the recovery stage. This review will make significant contributions to the Sustainable Development Goal (SDG), Goal 3, which ensures healthy lives and promotes well-being for all at all ages. We expect that the pooled empirical evidence will enable public health practitioners to collaborate with therapists to develop effective interventions and support services for BC patients to promote PTG among patients with trauma and PTSD.

## MATERIALS AND METHODS

### Study design

Empirically peer-reviewed articles from databases were pooled using the scope review approach. During the conceptualization of this topic, selected databases were screened for existing scoping reviews like the current review, but none were found. The scoping review adhered to the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) guidelines.

### Search strategy

Peer-reviewed articles published from 1999 to 2024 were identified from databases, including Web of Science, PubMed, Scimago, and PsycINFO. We developed search terms based on the key variables in the topic. Search terms developed from the link between trauma and BC included "BC pains" "BC distress" "BC shocks", "BC menace", and "breast terrifying experience". For the link between BC and PTSD, we used the following search terms: "BC patients' experiences of PTSD", "association between BC and PTSD", and "comorbid BC and PTSD". For the link between BC and PTG, we formulated the following search terms: "BC with comorbid PTG" and "PTG in women with BC". The literature search was concluded in March 2024.

### Eligibility criteria

**Inclusion Criteria:** All studies included in this review were peer-reviewed and empirical research articles. Studies conducted in the English language that used qualitative, quantitative, mixed-methods research approaches, and studies conducted exclusively on women from SSA women diagnosed with BC, were included.

**Exclusion criteria:** We excluded studies such as gray literature (theses or dissertations, book chapters, editorials, and conference abstracts), as well as all kinds of reviews and meta-analyses. Studies that addressed BC without trauma, PTSD, or PTG were excluded. In addition, studies that addressed trauma, PTSD, and PTG without BC were excluded.

### Study screening

**Screening and selection:** At this stage, the two-selection process included abstract and full-text screenings. In addition to conducting extensive searches of selected databases, a co-author with a strong background in educational psychology independently screened the titles and abstract sections of the selected articles to determine if they met the criteria for full-text screening. For possible screening, the first author, who has a background in educational research, reviewed all the references in the article. The two authors screened each article in entirety. The two authors used consensus to determine article inclusion after extensive discussion of the differences. First, the authors coded the data for data extraction. The screening process is represented in the PRISMA flow diagram in Figure 1.

### Data extraction process

Data were extracted from selected articles using a PICO-defined data extraction guideline. Data extracted from the literature include year, author name, country, population/sample, method, and results. The authors participated in the review. See Table 1.

## RESULTS

The scoping review search produced 769 records from the databases. After removing duplicate articles, 507 titles and abstracts were screened, and 185 articles were eliminated. In total, 322 full articles were assessed for eligibility, of which 298 were removed. Finally, 24 articles were included in the study, as summarized in Figure 1.

**Table 1 Features of the articles reviewed**

| Ref. | Location | Variables | Participant characteristics | Method/sample | Instruments | Results |
|---|---|---|---|---|---|---|
| Bosire *et al*[27], 2020 | South Africa | BC<br>Comorbid suffering | | Qualitative study<br>50 women | Interview | This study revealed that participants experienced discrimination and isolation, as well as fear of been rejected by their families. It was also found that BC patients are dissociated from their family members and the wider community |
| Maree *et al*[28], 2015 | Zambian | BC<br>Severe suffering | 48.2 years | Qualitative descriptive survey<br>10 participants | Interview | This qualitative study revealed that patients with advanced BC experience severe suffering before diagnosis. After undergoing chemotherapy, the patient became fearful of stigma and lost their femininity, strength, appearance, role, and support system |
| Lambert *et al*[29], 2020 | South Africa | BC<br>Trauma | Aged between 28 and 76 years. Average 49 | Qualitative<br>50 black women | Interview | This study revealed that most patients felt that they would die once diagnosed with cancer. Participants reported that chemotherapy causes fear, distress, and pain, which leads to traumatization |
| Coetzee *et al*[30], 2019 | South Africa | Breast treatment and experiences<br>Distress | Age between 48 and 66 years | Qualitative. 12 South African women | Interview | South African women react to BC with shock and disbelief. Women's experiences of diagnosis and treatment were accompanied by psychological distress |
| Teye-Kwadjo *et al*[31], 2022 | Ghana | BC<br>Persistent pain, physical appearance | Between 22 and 69 | Qualitative<br>12 Ghanaian women | Interview | Participants revealed that BC treatment and diagnosis are associated with chronic pain in the breast, chest, and armpit areas. It was revealed that participants feared loss of hair, swollen hands, and numbness due to treatment |
| Nwakasi *et al*[32], 2023 | Nigeria | BC<br>Cancer stigma | | Qualitative<br>30 BC survival | Interview | BC is a potentially stigmatizing illness |
| Iddrisu *et al*[33], 2020 | Ghana | BC<br>Trauma | From 28 to 45 years | Qualitative exploratory<br>12 participants | Interview | BC patients felt depressed, cried, and were traumatized after being diagnosed with BC. Some of the patients felt that they were unattractive due to the mastectomy done; however, they used handkerchiefs as breast prostheses |
| Lebimoyo and Sanni[34], 2023 | Nigeria | BC, PTSD | Between 25 to 60 years | Descriptive 183 patiently diagnosed female BCs | Questionnaires | Post-traumatic stress symptoms were 46% at baseline assessment. However, there was a significant reduction after 3 months (31%) and 6 months (22%). It was observed that PTSS is higher at early diagnosis |
| Eugenia *et al*[35], 2019 | Zimbabwe | BC<br>Anxiety, fear and depression, PTSD | Aged 30 to 80 years | Qualitative study<br>12 participants | Semi-structured interviews | 100% of participants experienced anxiety, 80% experienced post-traumatic stress, and 20% experienced depression |
| Alagizy *et al*[36], 2020 | Egypt | BC,<br>Trauma symptoms (anxiety, perceived stress, and depression). | Mean age 52.29 ± 11.64 years | Mixed method 60 BC patients | Questionnaires and interview | The study found that depressive symptoms, anxiety symptoms, and perceived stress were 68.6%, 73.3%, and 78.1% among patients, respectively |
| van Oers and Schlebusch[37], 2021 | South Africa | BC<br>Trauma symptoms (distress, suicidal ideation) | | Quantitative study. 80 female BC patients | Descriptive statistics<br>Questionnaires | BC patients experienced high levels of hopelessness and suicidal ideation. These patients also encounter psychological stress |
| Schlebusch and van Oers *et al*[38], 1999 | South Africa | BC<br>Trauma symptoms (psychological distress, hopelessness, and suicidal | Mean age for black 42.2 years and 54.3 years for white | Descriptive survey study. 50 South African women | Questionnaire | Black South Africans were found to experience depression, somatization, and body image dysphoria, and use less adaptive styles than white South Africans. As a result of BC symptoms, both groups experience the same level of anxiety |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | ideation) | | | | |
| Stecher *et al*[39], 2023 | South Africa | BC<br>Mastectomy | Age (34 to 58) | Qualitative 7 participants | Semi-structured interviews | Cultural stigma against BC patients still exists in the South African population |
| Ofei *et al*[40], 2023 | Ghana | BC<br>PTG | | 128 BC survival | Questionnaire | PTG among BC patients is determined by social supports, optimism, religiosity, and hope, all of which assist them in managing their illness |
| Aliche *et al*[41], 2023 | Nigeria | BC and other cancers<br>PTG | Age range 28–55 | 957 cancer patients | Questionnaire | In the Nigerian context, meaning in life, a mechanism of mindfulness was found to promote PTG in cancer patients |
| Olaseni *et al*[42], 2016 | Nigeria | BC and other cancers<br>PTG | Age range of 28–55 | 120 participants | Questionnaire | PTG was predicted by age, sex, education, and the results of the diagnosis |
| Aliche[43], 2022 | Nigeria | General cancer<br>PTG | | 550 patients | Questionnaire | Positive reappraisal and self-compassion independently mediated PTG. This indicates that reappraisal and self-compassion significantly facilitate PTG in patients with cancer |
| Gorven *et al*[44], 2018 | South Africa | BC<br>PTG | 25 and 50 years | Qualitative study<br>6 women | Interview | In South Africa, PTG involves losing body control, reclaiming body control, surrendering final control over the body, rebuilding personified identity, and gaining a new appreciation for the body |
| Agyei[45], 2018 | Ghana | BC<br>PTG | | Cross-sectional survey. 150 BC women | Questionnaires. | PTG was positively associated with age, survival year, and marital status. There was no association between educational level, religion, employment status, and PTG. It was also revealed that social support, coping, and optimism were directly related to PTG |
| Fekih-Romdhane *et al*[46], 2022 | Tunisia | BC<br>PTG | Mean age of 52.7 ± 9.8 | Quantitative seventy-nine (79) postoperative BC women | Questionnaires | Patients found that they were stronger than they assumed (70.0%), had strong religious faith (65%), and had the capacity to accept the way things work out (63.8%). The results also revealed that anxiety and social support are substantially associated with PTG |
| Njoroge and Asata *et al*[47], 2022 | Kenya | BC<br>Traumatic stress, and PTSD | Age group of 25–47 years | Mixed method research design. 60 females sampled through purposeful sampling | Impact of events scale revised and interviews | The structure of traumatic stress at the time of BC diagnosis and treatment depended on how test results were communicated to the patients, and treatment associated side effects like body image changes, mastectomy, and weight loss or gain. Also, 90% of the participants reported severe PTSD, while 6.70% and 3.30% reported moderate and low PTSD, respectively |
| Kagee *et al*[48], 2017 | South Africa | BC<br>Trauma symptoms (distress, and depression) | Mean age 55.70 years | Quantitative study. Sample of 201 female BC | Checklist Questionnaire | Distress and depression were prevalent among BC patients in South Africa, specifically those with higher body change stress and lower perceived support |
| Berhil *et al*[49], 2017 | Morocco | BC<br>Traumatic distress | Age 50 ± 8 | Quantitative sample of 446 Moroccan women | Questionnaire | A psychological distress prevalence rate of 26.9% was reported among Moroccan BC patients |
| Ohaeri *et al*[50], 2012 | Nigeria | BC,<br>Trauma symptom (psychic distress, and adjustment) | Age 49.9 | Descriptive research design. Sample of 63 attendees | Questionnaire | The greatest worry was associated with fear of death. Psychic distress was negatively associated with BC management. Fear of people's perceptions was a predictor of psychological distress |

BC: Breast cancer; PTSD: Post-traumatic stress disorder; PTG: Post-traumatic growth.

### *Characteristics of the included articles*

The features of the reviewed articles are presented in Table 1. The publication dates ranged from 1999 to 2023[27-50]. Fifteen studies investigated the comorbidity of trauma and BC, two studies treated BC and PTSD, and seven studies investigated the comorbidity of BC and PTG in Africa as depicted in Figure 2.

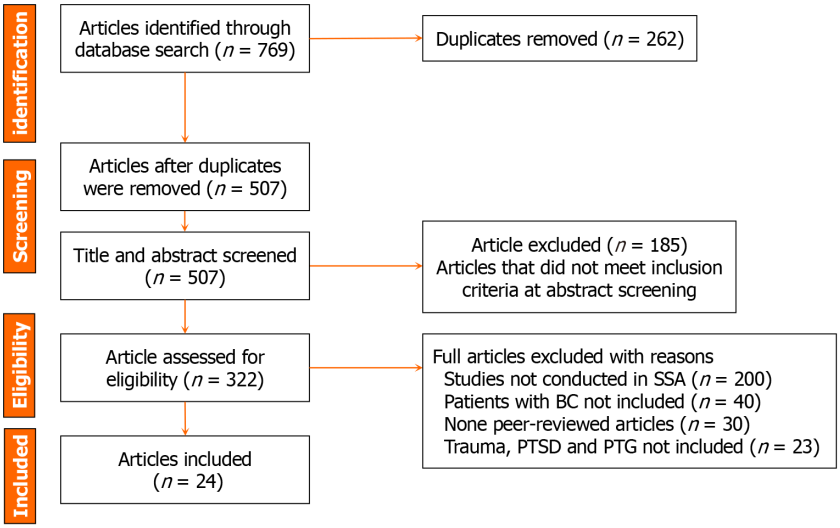


**Figure 1 PRISMA flow diagram demonstrating the article selection processes from identification to inclusion.** BC: Breast cancer; PTSD: Post-traumatic stress disorder; SSA: Sub-Saharan African countries.

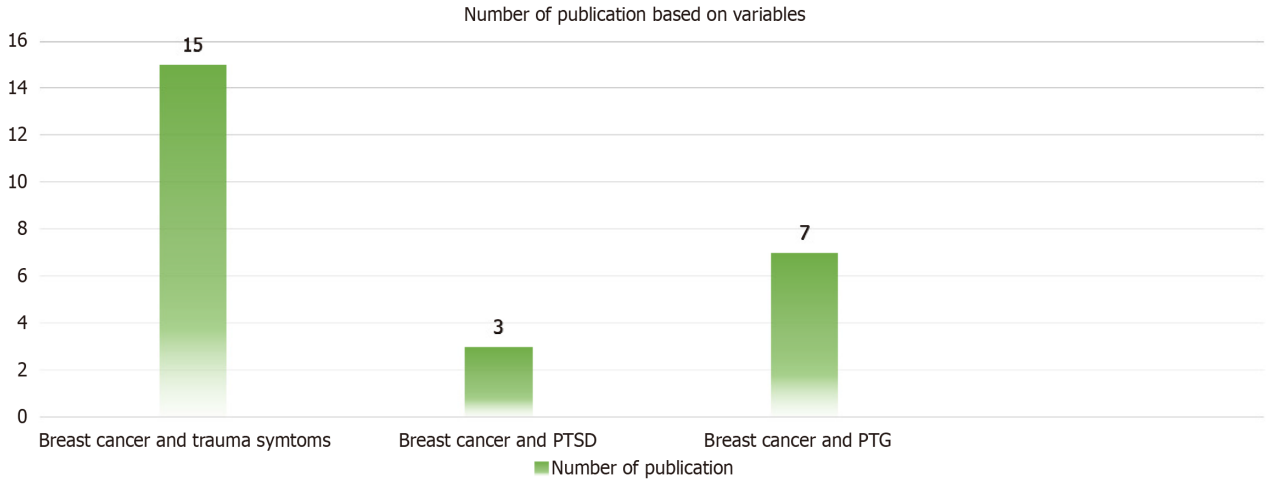


**Figure 2 Publications on breast cancer and trauma, breast cancer and post-traumatic stress disorder, and breast cancer and post-traumatic growth.** PTG: Post-traumatic growth; PTSD: Post-traumatic stress disorder.

Most studies were conducted in South Africa[27-30,37-39,44,48], followed by Nigeria[32,34,41-43,50], Ghana[31,33,40], Kenya[47], Morocco[49], Egypt[35], Zambia[28], Zimbabwe[35], and Tunisia[46], as shown in Figure 3. The first article selected for this review was published in 1999, while the most recent article was published in 2023 (Figure 4).

We included 3316 BC patients in this review. The comorbidity of trauma, PTSD, and PTG among BC patients gained the attention of research from 2017 until 2020, when there was a drastic decline in the interest of researchers in the area (Figure 4). However, there was a sharp increase in the interest of researchers from 2022 to 2023.

The major variables considered were trauma or trauma symptoms, PTSD, and PTG. The age of the participants in this review ranged from 25 to 80 years. Most of the studies adopted a quantitative[37,38,40-50], qualitative[27-33,39,44], and mixed method[36,47]. The instruments for data collection were interviews for qualitative studies and questionnaires for quantitative studies. However, some studies that adopted a mixed method utilized both interviews and questionnaires (Table 1).

### *Outcomes of the reviewed articles*

Table 1 reveals the major outcomes of the selected studies in this scoping review. These variables are organized into three major themes based on the three variables of the study, including the comorbidity of traumatic symptoms and BC ailment, PTSD and BC ailment, and PTG and BC (Figure 5). Fifteen studies investigated the comorbidity of BC and traumatic symptoms, revealing that BC patients experience traumatic events such as trauma associated with chemo-therapy treatment[29]; discrimination, isolation, and rejection[27]; severe pains before diagnosis[28]; loss of femininity [28]; stigmatization[39]; psychological distress associated with diagnosis and treatment[30]; fear of loss of hair; swollen hands and numbness due to treatment[31]; depression after being diagnosed with BC[33]; experiencing high levels of hopelessness and suicidal ideation; and that cultural stigma against BC patients still prevails within the South African

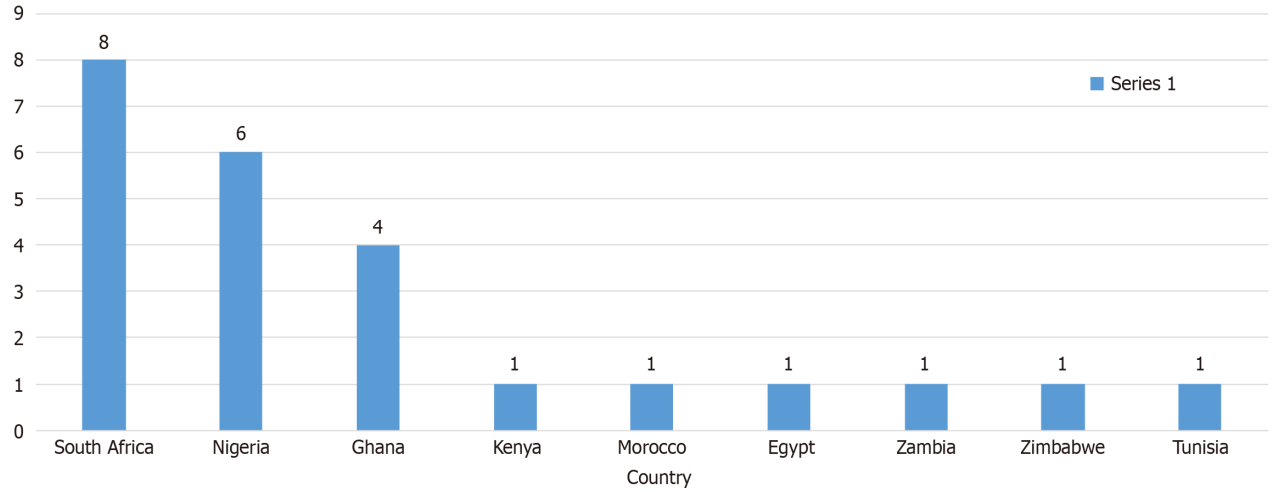


**Figure 3 Distribution of selected studies by country.**

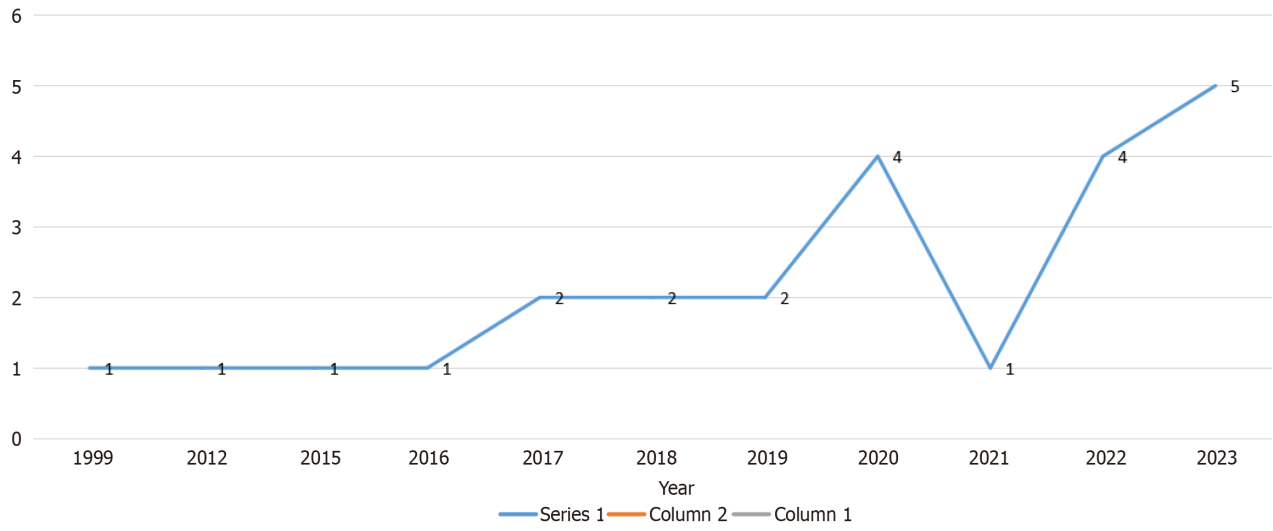


**Figure 4 Trend of publication on trauma, post-traumatic stress disorder, and post-traumatic growth based on year.**

population[39].

Studies have shown that the extent to which BC patients experience PTSD varies. In Kenya, a study found that 90% of the participants experienced severe PTSD, 6.70% experienced moderate PTSD, and 3.30% experienced low PTSD[47]. In Zimbabwe, a study revealed that 80% of BC patients reported PTSD[35]. In Nigeria, a study revealed that post-traumatic stress symptoms (PTSS) were 46% at baseline assessment and observed that PTSS is higher at early diagnosis[34]. The extent of PTSD experiences among BC patients during diagnosis and treatment in Africa depends on how the test results are communicated to the patients, and treatment associated side effects include body image changes, mastectomy, and weight loss or gain[47].

This review disclosed that PTG among BC patients in South Africa involves losing, regaining, surrendering final control over the body, rebuilding a personified identity, and newfound appreciation for the body[44]. In Nigeria and Ghana, PTS was promoted by social support, optimism, religiosity, and hope[41,45] as well as mindfulness[41]. In Tunisia, PTG was positively associated with strong religious faith (65%), and the potential to accept the way things work out (63.8%); however, it was negatively associated with anxiety. PTG among BC patients in Nigeria was mediated by positive reappraisal and self-compassion[43]. There is controversy over the predictors of PTG among BC patients in Africa. In Nigeria, a study found that educational levels of BC patients, knowledge of diagnosis, and religion predicted PTG among BC patients[42]. However, in Ghana, educational level, religion, and employment were not predictors[45].

## DISCUSSION

This scoping review is the first to examine existing empirical literature on the comorbidity of trauma, PTSD, and PTG among BC patients in Africa. Understanding the simultaneous occurrence of these psychological variables and BC

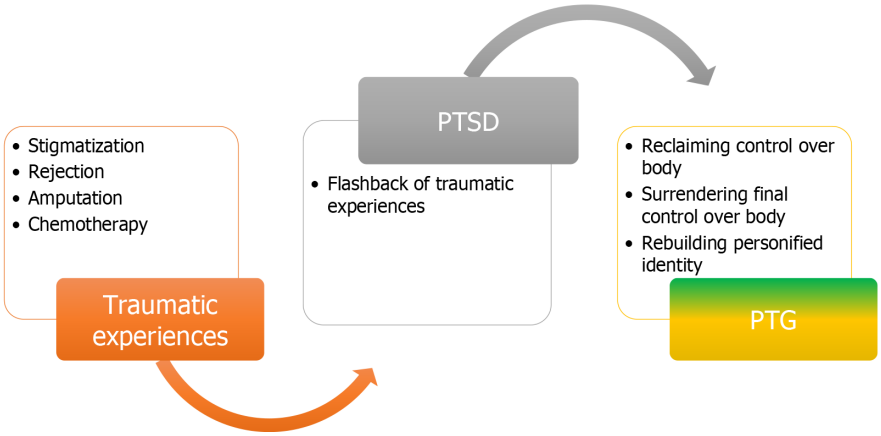


**Figure 5 Summary of findings.** PTG: Post-traumatic growth; PTSD: Post-traumatic stress disorder.

ailments in patients is crucial to guide future research and identify areas that need support and intervention. This is crucial since trauma, which is a psychological injury due to strong emotional stimulus, causes adverse mental health problems among BC patients. This discussion includes empirical evidence of the comorbidity of trauma and BC in patients, PTSD and BC, and PTG and BC.

With respect to the comorbidity of trauma and BC in patients, this study, through a systematic search for empirical evidence, revealed that BC patients experience several traumatic symptoms before diagnosis, during diagnosis, and during treatment in Africa**.** An amputation of the breast is one of the most common traumatic events experienced by BC patients in Africa. Amputation of the breast is linked to chemotherapy, which is characterized by excruciating pains that often lead to amputation of the breast. BC patients in Africa experienced chemotherapy, expressing fear of stigma and loss of femininity, appearance, and feminine roles due to amputation[27]. Studies have indicated that there is still a stigma associated with BC in Africa, and that family members, close friends, and the broader community discriminate against BC patients[27,39]. These experiences have resulted in the development of depressive symptoms, somatization, and body image dysphoria among African BC patients and survivors. In this severe scenario, traumatic experiences have engendered hopelessness, depression, and suicide ideation among patients and survival in the African region[37]. Briefly, the comorbidity of BC and traumatic experiences have devastating impacts on the mental health of BC patients in Africa, which needs immediate attention. This finding confirms the review reports of Freeney and Tormey[51] on BC and chronic pain. It was found in the study that BC is associated with chronic pain resulting from its symptoms and treatment, such as chemotherapy. The results of this review report confirm those of a scoping review conducted by Eseadi and Amedu[2] on BC patient depression experiences. The report from the scoping review revealed that depression is one of the common psychological problems that BC patients experience in Nigeria.

The results of this study indicate that PTSD and BC are comorbid among patients in Africa. This demonstrates that BC patients and survivors in Africa experience flashbacks of the painful experiences encountered at the time of diagnosis and treatment. However, the BC literature emphasizes that PTSD rates vary from country to country. Current evidence indicates that PTSD is high among Kenyan BC patients; 90% of Kenyan BC patients experience severe PTSD[47]. Zimbabwe came in second in the rankings for high incidence of PTSD among BC survivors, as a study found that over 80% of Zimbabwean BC patients were suffering from PTSD[35]. Nigeria took third place, as a study revealed that 46% of female BC patients experience PTSD at early diagnosis[34]. In Africa, BC patient experiences of PTSD are higher in early diagnosis, which is triggered by how the results of the diagnosis are communicated to patients. The manner in which BC patients receive their health information considers the severity of the ailment, deformation of the female body structure, and treatment implications to determine the degree of PTSD at later stages.

This scoping review revealed that PTSD experiences are not high among BC patients in Africa, as detailed in only three literature reports. The experience of PTSD among African BC patients has a substantial adverse impact on their mental health. Since the literature has emphasized that BC incidence is high at early diagnosis, early intervention should be emphasized among traumatized patients. This could be attributed to the low survival rate of BC patients, the lack of researcher interest in investigating the incidence of PTSD in BC patients, or the impact of support services that buffer the negative impact of traumatic experiences on developing PTSD. This finding confirms those of the systematic literature conducted by Parikh[26]. The study reported that PTSD is present at the early diagnosis of BC among patients, while PTG develops once treatment is initiated. The prevalence rate of PTSD among BC patients is higher than the results of the meta-analysis done by Wu *et al*[52], which revealed that the pooled incidence of PTSD among BC patients is 9.6%, and young adults are at a higher risk of developing PTSD.

Finally, this review found that BC patients in Africa experience PTG, which is the positive change individual patients experience after encountering traumatic events associated with BC treatment. PTG manifests in BC patients in Africa through losing the body, reclaiming control over the body, surrendering final control over the body, rebuilding a personified identity, and newfound appreciation for the body[41,45]. It is important to note that BC PTG is a complicated process of transmission from negative feelings that arise because of traumatic events to positive feelings of acceptance of changes as a result of the cancer diagnosis. Therefore, the PTG among BC patients in Africa centers on the total and significant conception of body structure. Several factors have been identified in the literature that promote PTG among

BC patients in Africa. However, these factors vary across African countries. The common facilitators of PTG among BC patients in Africa are strong religious faith, social support, educational status, and the individual potential to accept the way things unfold[40,42,46]. Religion and education have been identified as significant variables that promote PTG among BC patients in Africa due to the fact that most African countries are spiritually inclined and BC patients attach their medical treatment and recovery to their faith in God. However, there is still controversy among public health researchers in BC literature that some of these variables, such as religion and educational level, do not contribute to PTG in African patients. Therefore, we call on future researchers to investigate the variables that promote PTG among BC patients on the African continent and to resolve the lingering disagreement in the BC literature on the effectiveness of religiosity and education in enhancing PTG.

In the context of BC literature, this finding supports those of previous studies. The study conducted by Casellas-Grau *et al*[53] revealed that PTG among BC patients is positively associated with spirituality, hope, and meaning, and negatively associated with depressive and anxious symptoms. In addition, this supports a global review done by Kolokotroni *et al* [54], which revealed that PTG is related to social support, cognitive processing of BC, and coping strategies of BC women.

### *Knowledge gap in the literature*

This review has identified some significant knowledge gaps in research on traumatic, PTSD, and PTG experiences among BC patients in the African context. Most of the studies included in this review were conducted in South Africa, followed by Nigeria, and Ghana. This implies that research on the comorbidity of traumatic experiences, PTSD, and PTG is still growing. This is because few studies were retrieved from north, east, and central African countries. This infers that researchers within these locations are not aware of the devastating impact of the comorbidity of trauma and BC on the mental health of patients. Concerning the adverse effect of comorbidity in PTSD and BC patients, only three studies were conducted in this regard, while only seven studies revealed the PTG of BC patients. We call for more empirical studies in these areas to unveil recent empirical evidence on the experiences of trauma, PTSD, and PTG among BC patients in Africa.

### *Strengths and limitations of the review*

This review is the first to establish the trajectory of traumatic experiences, PTSD, and PTG among patients on the African continent. This review was able to explore several databases regarding traumatic experiences peculiar to BC patients in Africa. In addition, this review extended the scope of the literature to cover PTSD, and PTG among BC patients in Africa. We did not employ date restriction; hence, we revealed holistic empirical evidence concerning BC patient experiences of traumatic events, PTSD, and PTG in an African context. As strong as this review is, it has some inherent limitations. The review was limited to empirical literature published in English only, and other exclusion criteria were employed in the review process. The search terms used to explore the literature may have also contributed to the categories of studies retrieved for this review. These criteria may have excluded significant information that concerns the traumatic events, PTSD, and PTG experiences of BC patients in the African context. It should be noted, however, that these limitations do not invalidate the contribution of this review to the existing BC literature regarding the lives of African BC survivors with respect to their experiences of traumatic events, PTSD, and PTG.

## CONCLUSION

The comorbidities of trauma, PTSD, and BC have adverse effects on patient mental health; however, PTG promotes positive self-perception change. Our study revealed that BC patients in Africa experience many traumatic experiences that limit their recovery time, alter their self-perception, and change their role in society. In addition, empirical evidence has demonstrated that few of these BC survivors in Africa experience PTSD, which has a detrimental effect on their mental health. However, BC literature has revealed that some patients develop PTG after experiencing traumatic events, which is positively associated with recovery. In the Africa context, PTG has been associated with patients' faith in God, support services, and knowledge of the BC ailment. It is significant to emphasize the fact that BC patients, despite their encounter with traumatic events during diagnosis and treatment, also experience stigmatization and discrimination. This review has shown that the literature on trauma, PTSD, and PTG among BC patients in Africa is still growing; hence, more research needs to be done on the continent. We call for more research on the traumatic events associated with chemotherapy treatment and PTSD experiences in the African context. Researchers in Africa have underemphasized the experience of PTSD in BC patients. In light of the fact that BC is associated with psychological problems such as trauma and PTSD, we advocate for more intervention programs to curtail the adverse effects on patients. Furthermore, we call for a more collaborative approach to providing support services that will facilitate the PTG of BC patients in the African context.

## FOOTNOTES

**Conflict-of-interest statement:** No conflicts of interest.

**PRISMA 2009 Checklist statement:** The authors have read the PRISMA 2009 Checklist, and the manuscript was prepared and revised according to the PRISMA 2009 Checklist.

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**S-Editor:** Liu H
**L-Editor:** Filipodia
**P-Editor:** Wang WB

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World Journal of Experimental Medicine

Submit a Manuscript: https://www.f6publishing.com

*World J Exp Med* 2024 September 20; 14(3): 96720

DOI: 10.5493/wjem.v14.i3.96720

ISSN 2220-315x (online)

LETTER TO THE EDITOR

# Hypoglycaemia in screening oral glucose tolerance test in pregnancy with low birth weight fetus

Nicoleta Gana, Iulia Huluta, Nicolae Gica

**Specialty type:** Medicine, research and experimental

**Provenance and peer review:** Invited article; Externally peer reviewed.

**Peer-review model:** Single blind

**Peer-review report's classification**
**Scientific Quality:** Grade B
**Novelty:** Grade B
**Creativity or Innovation:** Grade B
**Scientific Significance:** Grade B

**P-Reviewer:** Yin CH

**Received:** May 13, 2024
**Revised:** June 30, 2024
**Accepted:** July 17, 2024
**Published online:** September 20, 2024
**Processing time:** 107 Days and 8 Hours

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## Abstract

Maternal hypoglycemia, a condition characterized by lower than normal blood glucose levels in pregnant women, has been increasingly associated with adverse pregnancy outcomes, including low birth weight (LBW) in neonates. LBW, defined as a birth weight of less than 2500 g, can result from various factors, including maternal nutrition, health status, and metabolic conditions like hypoglycemia. Maternal hypoglycemia may affect fetal growth by altering the supply of essential nutrients and oxygen to the fetus, leading to restricted fetal development and growth. This condition poses significant risks not only during pregnancy but also for the long-term health of the child, increasing the likelihood of developmental delays, health issues, and chronic conditions later in life. Research in this area has focused on understanding the mechanisms through which maternal hypoglycemia influences fetal development, with studies suggesting that alterations in placental blood flow and nutrient transport, as well as direct effects on fetal insulin levels and metabolism, may play a role. Given the potential impact of maternal hypoglycemia on neonatal health outcomes, early detection and management are crucial to minimize risks for LBW and its associated complications. Further investigations are needed to fully elucidate the complex interactions between maternal glucose levels and fetal growth, as well as to develop targeted interventions to support the health of both mother and child. Understanding these relationships is vital for improving prenatal care and outcomes for pregnancies complicated by hypoglycemia.

**Key Words:** Glucose tolerance test; Low birth weight; Hypoglycaemia; High-risk pregnancy; Neonatal outcome

**Core Tip:** Maternal hypoglycemia's association with low birth weight is a complex and nuanced issue that has garnered attention in the medical community. Emerging studies suggest a correlation between maternal blood glucose levels and birth weights, highlighting that not only hyperglycemia, but also hypoglycemia could significantly impact neonatal outcomes.

**Citation:** Gana N, Huluta I, Gica N. Hypoglycaemia in screening oral glucose tolerance test in pregnancy with low birth weight fetus. *World J Exp Med* 2024; 14(3): 96720
**URL:** https://www.wjgnet.com/2220-315x/full/v14/i3/96720.htm
**DOI:** https://dx.doi.org/10.5493/wjem.v14.i3.96720

## TO THE EDITOR

Neonatal low birth weight (LBW) presents a significant public health challenge worldwide. The World Health Organization estimated that in 2015, approximately one out of every seven live births globally, amounting to 20.5 million infants, were born with LBW. Furthermore, LBW is associated with over 80% of neonatal fatalities, with preterm births accounting for around two-thirds of these deaths[1]. Pregnancy hypoglycaemia arises from a relatively enhanced state of insulin action, which could be due to heightened insulin levels or an increase in insulin receptor activity, as well as a reduction in certain hormones that typically counteract insulin's effects, such as placental lactogen[2,3].

While women with gestational diabetes mellitus (GDM) often have elevated glucose levels, leading to a higher chance of birthing neonates that are large for gestational age, women experiencing low blood sugar may have a heightened risk of delivering neonates with LBW[4]. However, there has been limited research on the connection between low blood sugar during the oral glucose tolerance test (OGTT) and the subsequent effects on maternal and neonatal health, particularly in women who are normally glucose tolerant (NGT)[5,6]. Previous studies have mainly concentrated on the consequences of reactive hypoglycemia following an OGTT, with inconsistent findings regarding its influence on maternal and neonatal outcomes, such as neonatal birth weight. Furthermore, the bulk of this research has focused on hypoglycemic effects in women with GDM or obesity[7,8]. There is a lack of information on the pregnancy outcomes for NGT women who have lower or flat glycemic levels during the OGTT conducted between the 24th to 28th weeks of gestation[4,9]. A study published in 2022 concluded that hypoglycemia in OGTT is not associated with maternal or neonatal adverse outcomes[7].

Research into maternal hypoglycemia and its potential link to LBW is revealing important insights into fetal development. Unlike the well-established risks associated with GDM, where elevated maternal glucose can lead to large-for-gestational-age infants, maternal hypoglycemia represents a less understood risk factor[2,3].

We have read with great interest this observational study by Nayak *et al*[10] They explored the association between low glycemia during OGTT test and LBW. LBW was defined as below 2500 g and glycaemic markers were diagnostic for GDM as follows: Fasting glucose ≥ 5.6 mmol/L and/or a 2 h plasma glucose post 75 g glucose load ≥ 7.8 mmol/L. Hypoglycemia was defined by a blood glucose value ≤ 3.5 mmol/L[10]. This study has a large number of cases analyzed ( $n$ = 3537) on a 4 year period. They selected high risk women for GDM that underwent OGTT[10].

According to the authors, LBW was significantly higher in women with low plasma glucose when compared to women who delivered normal or macrosomic neonates. They have also stated that maternal age is not a significant factor[10].

The developing fetus relies on maternal glucose as its primary energy source, transported across the placenta without the need for insulin[9,11]. When a mother experiences episodes of hypoglycemia, this vital supply of glucose to the fetus may be compromised, potentially impeding fetal growth and development, leading to LBW and higher risks of admission into neonatal intensive care unit[12,13].

Most of the current literature focuses on the effects of hypoglycemia in populations of women with GDM or obesity, with scant data on otherwise healthy women with naturally lower glucose levels. This gap signifies a need for more robust, large-scale studies to better understand the potential impacts of maternal hypoglycemia on fetal growth and to establish clear clinical guidelines for managing low blood sugar levels during pregnancy to optimize neonatal outcomes.

Although studies show inconsistent data, it is important to have a different approach on women with low glucose levels at OGTT, given the fact that a large number of factors can contribute to LBW such as genetic conditions, structural abnormalities, and environmental factors[14].

So far most of the international societies recommends screening for GDM only to high risk women, it is important to think about having all women screened at 24-28 weeks not only for GDM, but also for prediction of adverse perinatal and neonatal outcomes[15].

Finally, the authors well presented, on a large number of cases, an association of low fasting plasma glucose and a low glucose response at OGTT with LBW in high risk women for GDM screened at 24-28 weeks and highlights the importance of hypolgycaemia, which otherwise it is considered to be normal.

## FOOTNOTES

**Author contributions:** Gana N, Huluta I writing and revision; Gica N concept and supervision.

**Conflict-of-interest statement:** All the authors report no relevant conflicts of interest for this article.



**Country of origin:** Romania

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**S-Editor:** Liu H
**L-Editor:** A
**P-Editor:** Chen YX

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