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ABOUT COVER

Editorial board member of *World Journal of Diabetes*, Dr. José Carvalheira is an Associate Professor at the Campinas State University (UNICAMP, Brazil). Having received his Bachelor's degree from Pernambuco University College of Medicine in 1994, Dr. Carvalheira undertook his postgraduate training at UNICAMP, receiving his PhD in 2004. He became Chief Physician in the Clinical Oncology Division of the Hospital de Clínicas Affiliated to UNICAMP in 2014 and has held the position since. His ongoing research interests involve investigation of how body composition, energy homeostasis, and the underlying mechanisms of whole-body metabolism are linked to cancer survival outcomes. In particular, his studies aim to elucidate the regulatory role of insulin resistance and its attendant pathophysiological features in determination of cancer-mediated disturbances in systemic homeostasis and cancer prognosis. (L-Editor: Filipodia)

AIMS AND SCOPE

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Type 1 diabetes mellitus and its oral tolerance therapy

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Abstract

As a T cell-mediated autoimmune disease, type 1 diabetes mellitus (T1DM) is marked by insulin defect resulting from the destruction of pancreatic β-cells. The understanding of various aspects of T1DM, such as its epidemiology, pathobiology, pathogenesis, clinical manifestations, and complications, has been greatly promoted by valuable research performed during the past decades. However, these findings have not been translated into an effective treatment. The ideal treatment should safely repair the destroyed immune balance in a long-lasting manner, preventing or stopping the destruction of β-cells. As a type of immune hypo-responsiveness to the orally administrated antigen, oral tolerance may be induced by enhancement of regulatory T cells (Tregs) or by anergy/deletion of T cells, depending on the dosage of orally administrated antigen. Acting as an antigen-specific immunotherapy, oral tolerance therapy for T1DM has been mainly performed using animal models and some clinical trials have been completed or are still ongoing. Based on the review of the proposed mechanism of the development of T1DM and oral tolerance, we give a current overview of oral tolerance therapy for T1DM conducted in both animal models and clinical trials.

Key Words: Type 1 diabetes mellitus; Immunotherapy; Oral tolerance; Regulatory T cells; Antigen-specific immunotherapy

Core Tip: As an antigen-specific immunotherapy, oral tolerance therapy has shown promise as a new strategy for the prevention and treatment of autoimmune diseases, including type 1 diabetes mellitus. Oral tolerance therapy in type 1 diabetes mellitus has been studied widely for a long time. In order to give a better understanding of these studies performed in animal models as well as in clinical trials, we review the related reports carefully and divide these studies into various categories based on their strategies. This careful review may be useful to guide the future studies.

INTRODUCTION

Based on the recommendations of the American Diabetes Association, type 1 diabetes mellitus (T1DM) includes two types: Idiopathic diabetes and immune-mediated diabetes[1]. The etiology of idiopathic diabetes is not known. The related patients bear perpetual insulinopenia and show a trend to ketoacidosis, however, autoimmunity is not observed. Resulting from T cell-mediated attack of insulin-producing β-cells of the pancreas, immune-mediated diabetes was previously termed T1DM, insulin-dependent diabetes, or juvenile-onset diabetes[1]. In this review, T1DM is used to refer to immune-mediated diabetes.

TYPE 1 DIABETES MELLITUS

As an autoimmune disorder, T1DM is marked by T cell-mediated destruction of β-cells of the pancreas, which leads to an almost complete loss of the ability to synthesize insulin[2]. This insulin deficiency results in loss of the ability to regulate blood sugar, therefore, exogenous insulin administration is necessary for patients to control blood sugar and to reduce the incidence of related chronic diabetic complications, such as the microvascular, macrovascular, and neuropathic complications[3]. The incidence and prevalence of T1DM have been growing all over the world and the relative annual increase in T1DM incidence is approximately 2%-3%[4,5]. The greatest increase is detected in children younger than 5 years[6]. T1DM can occur at any age[7] and therefore, the previous definition of T1DM as juvenile-onset diabetes may not be suitable.

During the development of T1DM, the autoimmune attack of β-cells of the pancreas mediated by T cells is regarded as the final step[8]. Typical T1DM related autoantibodies target various proteins, including (pro) insulin, glutamic acid decarboxylase (GAD) 65, Hsp60, IA-2, ZNT8, and tetraspanin-7[9-11]. Various autoantibodies can be observed many months or years prior to clinical onset (Figure 1). These autoantibodies can be considered biomarkers of β-cell injury rather than its cause. The mechanism triggering this autoimmune process remains elusive and this is the most crucial challenge for preventing T1DM. Studies to date propose that interactions between genetic susceptibility and environmental exposures (Figure 1, stage 0) are necessary in the development of T1DM. There have been identified over 60 genetic loci related to susceptibility to T1DM[12]. These loci can be divided to two types: HLA and non-HLA genes[13,14]. Combined with familial risk analysis, HLA risk analysis can be applied for identifying people at a high risk for T1DM[15,16]. However, individual non-HLA loci could not be applied for predicting T1DM or distinguishing it from other types of diabetes. To well predict the risk of developing T1DM and distinguish it from T2DM (type 2 diabetes mellitus), genetic risk scores derived from combined analysis of HLA and non-HLA genes can be applied[17,18]. Compared to the relatively well-known genetic factors, environmental determinants remain poorly understood despite intensive research. Studies to date show that diet (maternal diet, cow's milk protein, breastfeeding, gluten, and micro-

Stage	0	1	2	3
Autoantibodies	0-1	≥ 2	≥ 2	≥ 2
Dysglycaemia	Absent	Absent	Dysglycaemia	Hyperglycaemia
Symptoms	Absent	Absent	Absent	Present
Ability to predict T1DM	Less accurate	Less accurate	Highly accurate	Highly accurate
Prevention trial category	Primary prevention	Secondary prevention	Secondary prevention	Tertiary prevention (intervention)
Ability to prevent T1DM	More effective	More effective	Less effective	Less effective

Figure 1 Proposed staging of type 1 diabetes mellitus. T1DM: Type 1 diabetes mellitus.

and macro-nutrients), vitamin D sufficiency, virus infection, and the bacterial microbiome in the gastrointestinal tract (GIT) are associated with T1DM[2,16]. In addition, both genetic and environmental factors, particularly the latter, might influence the entire natural history of T1DM[13,19].

The establishment of β-cell autoimmunity can be confirmed by the occurrence of one or more autoantibodies as described above. Compared to individuals without autoantibodies, individuals with a single autoantibody may be at higher risk for T1DM. However, among people with a single autoantibody, the incidence of T1DM over 10 years is less than 10%[20,21]. If there are two or more autoantibodies present, the individual will eventually progress to clinical onset of T1DM[22] and this progression may be divided into three stages (Figure 1)[23]. Once two or more autoantibodies appear, it means that the individual has stage 1 T1DM. Apart from the number of autoantibodies observed and their seroconversion age, the type and affinity of autoantibody appearing in this stage contribute to the rate of progression to symptomatic T1DM[23]. However, the individual is normoglycemic and bears no clinical symptom in this stage. As the functional β-cell mass decreases, the individual in stage 2 develops dysglycaemia or glucose intolerance. Similar to stage 1, there is no obvious clinical symptom in stage 2 and therefore, stages 1 and 2 can be termed "presymptomatic T1DM". As the disease progresses, the functional β-cell mass has been severely impaired, leading to the inability to maintain normal metabolic function. Therefore, hyperglycaemia and typical clinical symptoms of diabetes (polyuria, polydipsia, and weight loss) occur and T1DM can be clinically diagnosed in stage 3 (symptomatic T1DM)[24]. At the time of diagnosis, there is approximately 80% to 90% of the original β-cell mass disrupted[25]. From a few weeks to up to 20 years will be needed to progress from the presence of autoantibodies to clinical onset and this prediabetic phase facilitates to predict and prevent or delay T1DM[16,26,27]. For prevention of T1DM, similar to other autoimmune diseases, it is more effective during the early stages (stage 0, primary prevention or stage 1, secondary prevention) of disease progression[24,28]. However, compared to stages 2 and 3, prediction strategies performed in stages 0 and 1 show a lower accuracy[26,28]. This imbalance between the predictive and preventive effect may lead to the fact that there has been a substantial loss of functional β-cell mass when T1DM is predicted accurately, which makes various therapies less effective in preventing, delaying, or reversing T1DM. Currently, few studies to successfully prevent the onset of T1DM have been reported[24,29].

ORAL TOLERANCE

Oral tolerance represents an adaptation, unresponsiveness, or hypo-responsiveness to an orally administered antigen by the immune system. Oral tolerance prevents inappropriate immune responses to innocuous antigens contained in food or commensal organisms. Thus, this process functions importantly in maintaining a balance between reactions against these exogenous antigens and self-components of the body in the gut mucosa[30]. Once this balance breaks down due to the disruption of oral tolerance, immunoglobulin E (IgE)-mediated food allergies, inflammatory bowel disorders, autoimmune disorders, or infections may occur[31]. As a physiological response to dietary antigens, oral tolerance mainly develops in the GIT. Although most of the orally delivered antigens are digested into short peptides and/or amino acids, a small amount of intact antigens can reach the intestinal epithelium where they are delivered in various manners, depending on their characters (*e.g.*, size and solubility). The delivery manner affects the subsequent immune response including the induction of tolerance or immunity[32].

The delivery of antigens contained in the gut lumen involves complex processes and there are various possible routes for antigens passing through the intestinal epithelium as shown in Figure 2. Soluble antigens might be delivered by enterocytes in two nonspecific manners, including the transcellular manner and the paracellular manner[33]. For the transcellular manner, antigens contained in vesicles may be degraded and however, some intact antigens can reach the basolateral space. For the paracellular manner, it may be suppressed under homeostatic conditions as a result of the tight junctions between enterocytes. The neonatal IgG receptor (FcRn), which locates on the surface of enterocytes, may be used to capture and internalize antigens by forming the antigen-antibody complexes[34]. And then, antigens are delivered across the epithelium to lamina propria dendritic cells (DCs) bearing FcRn as well as other Fc receptors. Antigens contained in the apoptotic enterocytes can be captured by neighbouring DCs. Both CX3CR1+ macrophages and CX3CR1+ DCs can sample luminal antigens efficiently by means of extending their dendrites between enterocytes without disrupting the integrity of the epithelium[35,36]. The above DCs or macrophages have no capacity to transport antigens directly to naïve T cells that locate in the mesenteric lymph nodes (MLN). However, they can transport these antigens to neighbouring CD103+ DCs, which subsequently enter the MLN, *via* a CC-chemokine receptor 7-dependent mechanism, and present antigens to naïve T cells[36,37]. In addition, mucus-secreting goblet cells perform an important role in delivering soluble antigens *via* goblet cell-associated antigen passages, which provide a conduit for delivering soluble antigens exclusively to CD103+ DCs and therefore, this process may perform a critical role in oral tolerance induction[38]. Located in the epithelial layer covering Peyer's patches (PPs), microfold cells (M cells) can specially deliver antigens including particles, bacteria, and viruses to CD103+ DCs that reside within the M cell pocket[39]. However, M cells can also be found outside PPs to deliver antigens[40]. Thus, the uptake of antigens through the intestinal epithelium is a complicated process. Currently, various routes as descried above have been suggested, however, this still is a disputed topic and needs to be further investigated.

Composed of the MLN and PPs, gut-associated lymphoid tissue (GALT) functions heavily in inducing oral tolerance successfully. Based on those proposed delivery routes as described above, CD103+ DCs harboring antigens derived from the gut lumen move into the MLN[41], which has been identified as an important site of oral tolerance induction[42]. However, compared to the MLN, PPs may not be necessary for oral tolerance induction[43,44]. In the MLN, CD103+ DCs harboring antigens meet naïve CD4+ and CD8+ T cells and oral tolerance induction occurs in an antigen dose-dependent manner, including low- and high-dose tolerance[33,45]. With regard to repeated feeding of a low dose of antigens (Figure 2), the generation of regulatory T cells (Tregs) helps to induce oral tolerance. Derived from CD103+ DCs harboring antigens in the MLN, indoleamine 2,3-dioxygenase, retinoic acid, and transforming growth factor-beta (TGF-β) appear to cooperate to facilitate naïve T cells differentiation towards Tregs which bear the gut-homing-associated adhesion molecules, such as chemokine receptor CCR9 and α4β7 integrin[33,39]. Therefore, these induced antigen-specific Tregs, which perform suppressor activity, can return into the lamina propria in which they are expanded with help of interleukin-10 (IL-10) secreted by CX3CR1+ macrophages. Both the induced Tregs' gut-homing and expansion are essential to achieve oral tolerance successfully[46,47]. The best-described Tregs are CD4+CD25+Foxp3+ Tregs which function a lot in oral tolerance induction, and this can be supported by the observation that specific depletion of Foxp3+ cells appears to abrogate oral tolerance[47]. Apart from CD4+CD25+Foxp3+ Tregs, other peripherally induced Tregs,
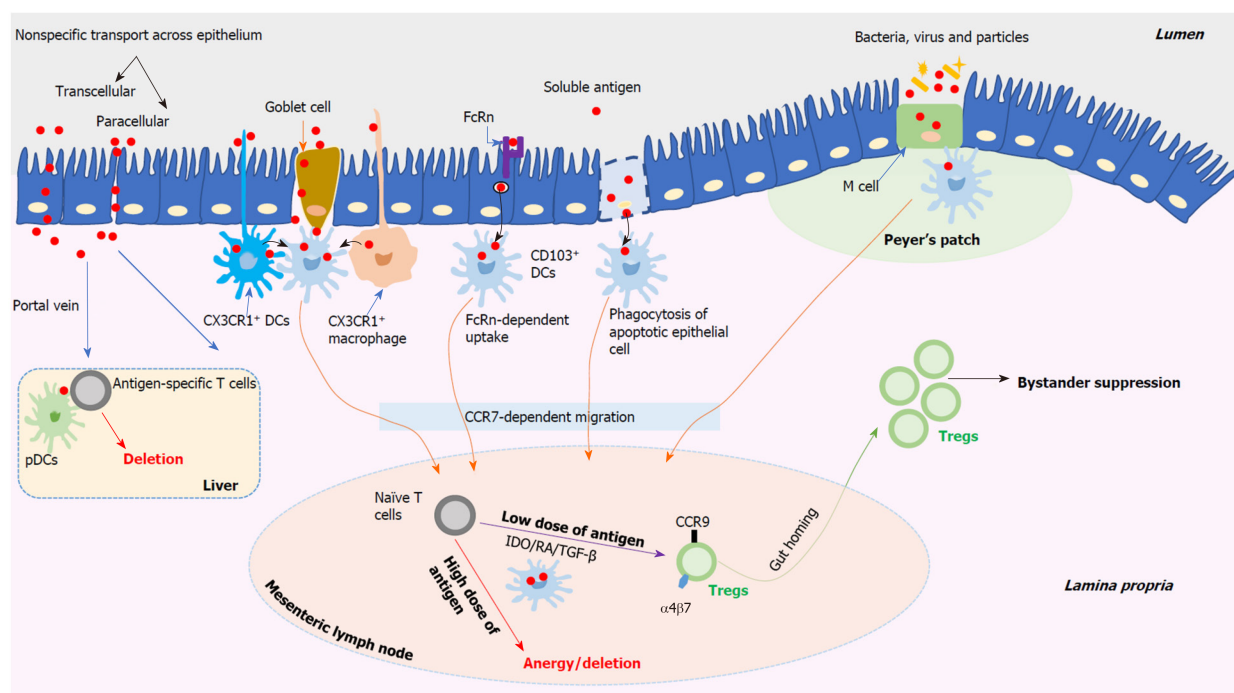
Figure 2 Schematic overview of the stepwise mechanisms of oral tolerance induction. DCs: Dendritic cells; M cell: Microfold cell; CCR: CC-chemokine receptor; pDCs: Plasmacytoid DCs; IDO: Indoleamine 2,3-dioxygenase; RA: Retinoic acid; TGF-β: Transforming growth factor-beta.

such as T helper (Th) 3 cells and type 1 regulatory T (Tr1) cells, are also related to oral tolerance induction[33,37]. All these peripherally induced Tregs contribute to inducing systemic immune tolerance by means of cytokine-mediated bystander suppression which is induced by oral feeding with one antigen and then works in a non-antigen-specific manner[30,48,49]. However, the potential function of Tr1 cells in oral tolerance induction and the potential relationship or interaction between these different Treg subsets are not clear and need to be further studied. Transferring T cells from tolerized animals obtained by feeding a low dose of antigens can transfer antigen-specific tolerance to naïve animals[45,50,51].

In respect to single feeding of a high dose of antigens (Figure 2), anergy and/or deletion of antigen-specific T cells can be induced in GALT, which contributes to oral tolerance[30]. However, transferring T cells from tolerized animals obtained by feeding a high dose of antigens fails to transfer this tolerance to naïve animals[45,52]. Apart from the MLN, the liver provides a complementary effect in oral tolerance induction (Figure 2). The portal vein offers an access for the gut-derived antigens, which are not processed in the gut, to the liver in which the tolerogenic environment contributes to the induction of oral tolerance[53]. Mediated by plasmacytoid DCs, the deletion of antigen-specific T cells occurs and this induces systemic tolerance to these antigens[54]. In addition, the tonsils and sublingual mucosa, especially their contained DCs, may also contribute to developing oral tolerance[55,56]. An important role for antibodies has been suggested in oral tolerance induction[33,45]. IgE-mediated hypersensitivity can be suppressed by administration of IgG antibodies[57]. Peanut tolerance can be induced among high-risk children by early introduction of peanut, which is mediated by the production of peanut-specific IgG4[58]. However, the mechanisms how antibodies benefit for oral tolerance induction remains to be determined. Additionally, it has been proposed that the intestinal microbiota and diet, such as vitamin D[59], may perform a positive effect for oral tolerance induction[45]. Among those oral tolerance studies performed during the past decades, most have been designed and applied to prevent and/or treat food allergy and autoimmune diseases, including T1DM[37,39].

ORAL TOLERANCE IN T1DM

As described above, individuals at risk for T1DM may be identified years before clinical onset. This makes oral tolerance, which acts as an antigen-specific immunotherapy, an attractive treatment for preventing and/or delaying T1DM[60,61].

Since its first application in the non-obese diabetic (NOD) mouse[62], which shares various immunological and pathological similarities to human T1DM and has been used as the primary model for investigating T1DM[63], many attempts to induce antigen-specific tolerance in NOD mice by feeding various autoantigens have been reported. Apart from the NOD mouse, other animal models including BioBreeding (BB) rats and a genetically modified mouse model induced by the lymphocytic choriomeningitis virus (LCMV) were also applied in oral tolerance induction for autoimmune diabetes. In addition, oral tolerance trials have been applied in human T1DM based on the results in animals. In order to get a comprehensive understanding of the progress that has been made so far in oral tolerance induction for T1DM, reports related to these valuable trials in both animals and human are collected, classified, and reviewed.

Oral tolerance trials in animals

Based on the formula used, these trials can be divided into two types, direct oral administration of a single autoantigen and combinatorial therapy. The latter may be further divided into the following types: A combination of different autoantigens and a combination of autoantigen(s) with various delivery vehicles and/or immune adjuvants and/or immunomodulatory agents.

Direct oral administration of a single autoantigen

As described above, six autoantigens related to T1DM have been identified, however, only two of them have been applied for oral tolerance induction in animals by direct oral administration, including insulin (proinsulin) and GAD65. Furthermore, among these two autoantigens, insulin was the most often used one.

Insulin: In 1991, the first trial in oral tolerance therapy for T1DM in NOD mice was applied by feeding porcine insulin[62]. At 5 wk of age, NOD mice were administered porcine insulin orally two times weekly for 5 wk and then weekly until one year old. Compared to animals administered phosphate buffer saline, or 10 μg or 100 μg of porcine insulin orally, animals fed 1 mg of porcine insulin showed a delayed onset and a reduced incidence of T1DM. In following years, this research group continued to apply modified trials for oral tolerance induction in animal models and to explore the responsible mechanisms. Based on their research and reports, they have made an important contribution to clarifying the mechanisms of oral tolerance as described above[30,46,49,64]. In respect to oral tolerance induction for T1DM, equine insulin[65,66], the B-chain of bovine insulin (30 amino acids)[67,68], and human insulin and its B-chain peptide (10-24)[69,70] have been applied by this research group. All these trials showed a positive effect in delaying and/or suppressing the process of insulitis and T1DM in NOD mice. Furthermore, cell culture, cytokine assays, and adoptive transfer studies suggested that this protective effect was related to a T cell dependent mechanism in which Tregs secreting IL-4, IL-10, and TGF-β played a critical role. In this context, there was a decrease in Th1 responses (downregulation of IL-2 and interferon-gamma) and an increase in Th2 responses (upregulation of IL-4 and IL-10) to the antigen administered orally. These non-antigen-specific cytokines mediated bystander suppression to different autoantigens[64]. Also, they found that feeding insulin or its B-chain peptide to one-day-old neonatal NOD mice performed effectively in preventing T1DM rather than accelerating the progression of diabetes[69]. Apart from these reports from the above research group, many trials applied by other groups supported the positive effect of tolerance derived from oral administration of insulin towards suppressing autoimmune diabetes in animals. Diabetes can be actively suppressed in NOD mice by feeding 20 U of insulin every 2-3 d for a month beginning at 6 wk of age and this protective effect was related to insulin-specific regulatory CD4+ T cells which were induced in the gut and subsequently homed to the islets[71]. The same research group illuminated that insulin-reactive Tregs can be induced in the spleen by oral insulin and the induced Tregs preferentially migrate to the pancreas and pancreatic lymph nodes[72,73]. No matter applied before or after the induced autoimmune response, 1 mg of insulin administered orally twice weekly can suppress the disruption of β-cells and prevent diabetes in the LCMV induced mice, and the change of the profile of pancreatic cytokine expression from interferon-gamma to immunosuppressive ones (IL-4, IL-10, and TGF-β) was responsible for this protective effect[74]. However, one amino acid substitution in insulin ($B_{30}A$ to T) abrogated this protective effect in both NOD and LCMV induced mice, indicating that the structure and/or sequence of the applied antigens may significantly affect the efficiency of oral tolerance induction[75]. Simultaneously, this report indicated that oral tolerance induction was not dependent on hormonal activity of insulin and accordingly, β-cell

rest was not the reason why oral insulin may prevent or delay the onset of T1DM.

In addition to these above trials bearing a positive effect by direct oral administration of insulin, there were reports indicating no effect in preventing T1DM by feeding insulin or its related peptides to animal models. Oral bovine insulin at all applied doses (0.5, 1.0, and 2.0 mg/wk, 3 wk) had no effect in preventing or delaying autoimmune diabetes in BB rats and the species/strain-specific traits of disease progression may be responsible for this failure to tolerance induction in BB rats compared to NOD mice[76]. Beginning at 30 d of age, NOD mice received 1 mg oral human recombinant insulin twice weekly. The protective effect obtained from this treatment showed no significant difference from the control group although a delay of the onset of disease was detected[77]. An oral tolerance study repeated in 2016 showed that feeding NOD mice of 5 or 9 wk of age with various dosages of different types of insulin for 5 wk had no ability to prevent T1DM[78]. The source and purity of these used insulin, the efficiency of insulin digestion, or the variability in disease penetrance in NOD mouse colonies caused by geographical differences may be responsible for this inability to prevent T1DM, which is in stark contrast to those above trials bearing positive effects in preventing T1DM in animals by direct oral feeding of insulin.

GAD65: In addition to insulin, GAD65 was the other autoantigen applied for oral tolerance induction in animal models by direct oral administration and however, there was only one related report as described below. Oral administration of 0.5 mg porcine GAD65 twice per week beginning at 30 d of age showed disease preventive effects during a long research period (> 400 d), however, compared to the control group, no difference in the final disease incidence was observed (P = 0.226)[77].

Combinatorial therapy

On the basis of the effects of direct oral administration of single autoantigen on tolerance induction in trials described above, combinatorial therapy has been suggested by various reports to improve the tolerance induction efficiency and the preventive effect towards autoimmune diabetes in animals. In addition to combination of different autoantigens by direct oral administration, various combinatorial strategies have been applied. To protect autoantigens from being degraded when passing through the GIT and/or reduce the required amounts of the used autoantigens, plant-, microbe-, or nanoparticles (NPs)-based delivery vehicles have been developed. In addition, immune adjuvants and immunomodulatory agents have been applied to combination therapy with autoantigens.

Direct oral administration of different autoantigens: Compared to oral administration of porcine GAD65 or human recombinant insulin alone, combined therapy with both autoantigens performed significant anti-diabetic effects (P = 0.011). Splenocytes from mice treated with the combined therapy showed a reduced response to both porcine GAD65 and insulin, indicating that antigen-specific inhibition was generated[77]. In addition to the above report, there was no more report applying direct oral feeding with various autoantigens for tolerance induction in animals, however, oral feeding with different autoantigens has been applied in combination with various delivery vehicles and/or immune adjuvants, and this will be reviewed in the following sections.

Various vehicles applied for delivery of autoantigen(s) to induce oral tolerance: In 1997, GAD67, a GAD isoform predominating in mouse islets, was expressed in transgenic plants. Oral administration of these GAD-containing plants (approximately 1-1.5 mg of GAD daily) from 5 wk to 8 mo of age suppressed the development of T1DM in NOD mice[79]. However, oral therapy with tobacco expressing 0.5 mg human GAD65, once a week for 10 wk beginning at 5 wk of age, was not sufficient to protect the NOD mice from autoimmune diabetes[80]. Compared to the above study applying GAD67-expressing transgenic plants for oral tolerance induction, the low dosage and the short treatment course applied in this study may be responsible for the failure to prevent T1DM in NOD mice. Various strains of lactic acid bacteria (LAB), generally regarded as safe, have been tested as mucosal delivery vehicles for protein/DNA-based vaccines[81] and for tolerogenic immunotherapy[82]. Some T1DM autoantigens or their related peptides, such as proinsulin[83], HSP60[84], GAD65[85], and IA-2[86], have been expressed in recombinant *Lactococcus lactis* (*L. lactis*) and applied for antigen-specific tolerance induction in combination with some immunomodulatory agents to prevent or reverse T1DM in animal models. This will be discussed in the next section. In respect to its two-chain structure, the expression of insulin is challenging for *L. lactis* and therefore, the expression of single-chain insulin (SCI) analogue may be an

alternative strategy[87]. Containing a short peptide linker between two chains of insulin, SCI-57[88] and SCI-59[89] were successfully secreted in *L. lactis*, respectively, retaining their insulin receptor-binding ability. Apart from the genetically modified LAB, non-living LAB bacterium-like particles (BLPs), which perform less anti-carrier response when administrated orally, have been applied as immunostimulants and/or mucosal vaccine delivery vehicles[90]. Oral feeding with BLPs-SCI-59, in which the above SCI-59 is bound to LAB BLPs in a non-covalent manner, could suppress the process of autoimmune diabetes in NOD mice by restoring the Th1/Th2 imbalance and enhancing Tregs proportions[91].

Autoantigen(s) in combination with immune adjuvants and/or immunomodulatory agents: Based on the preventive effect towards T1DM by direct oral feeding of autoantigen(s) as described above, its therapeutic potential may be restricted by requiring repeated administrations of a large amount of autoantigens. To overcome such restrictions, the nontoxic cholera toxin B (CTB) subunit has been applied as a mucosal carrier molecule for the conjugated autoantigens to induce oral tolerance[92]. The proposed mechanism for applying CTB as an effective mucosal carrier may be associated with its high GM1 binding affinity, which facilitates the conjugated protein delivery and presentation into the GALT. In addition, CTB's immunomodulatory properties may also be responsible[92,93]. The incidence of disease can be reduced significantly in NOD mice even by feeding a single dose of minute amounts (2-20 µg) of CTB-insulin (CTB-INS) conjugate at 8 wk of age and a more durable effect can be obtained by applying five consecutive dosages of this CTB-INS conjugate[94]. This protective mechanisms by feeding microgram amounts of CTB-INS depend on the upregulation of Tregs which selectively home to the pancreas and its draining lymph nodes[95] and oral delivery of CTB-INS conjugates makes the precise sources of the applied insulin less important and reduces the required antigenic dosages[96]. At 5 wk of age, NOD mice were orally administered with transgenic potato harboring approximately 20 µg of CTB-INS conjugate (once per week for 5 consecutive weeks)[93]. Compared to administration of potato harboring insulin or CTB alone, feeding of the above tissues containing CTB-INS had significant suppression of autoimmune diabetes[93]. Beginning at 5 wk of age, oral feeding of transgenic plant tissues containing approximately 14 µg of CTB-proinsulin (CTB-pINS) protein (once a week for 7 consecutive weeks) alleviated pancreatic insulitis and significantly preserved pancreatic β-cells. And Th2 lymphocyte-mediated oral tolerance contributed to this beneficial effect[97]. However, beginning at 5 wk of age, feeding of transplastomic tobacco leaf material containing 25 µg, or 250 µg, or 500 µg CTB-human proinsulin (CTB-hpINS) once per week for 10 wk was not sufficient to prevent disease in NOD mice. Furthermore, no synergism was detected by oral feeding of tobacco tissues containing CTB-hpINS and GAD[80].

Using the silkworm as a bioreactor, edible vaccines containing CTB-INS, CTB-INS B chain, CTB-INS bearing a green fluorescent protein tag (CTB-INS-GFP), or CTB-INS fused to three copies of GAD65 peptide 531-545 (CTB-INS-GAD) were produced and applied to induce oral tolerance in NOD mice by one research group from Zhejiang University. Beginning at 5 wk of age, oral administration of 30 µg of CTB-INS or CTB-INS B chain (3-4 times one week for 5 consecutive weeks) can effectively prevent autoimmune diabetes in NOD mice[92,98]. Beginning at 5 wk of age, oral administration of 50 µg of CTB-INS-GFP (every other day for 5 consecutive weeks) induced specific immune tolerance and suppressed T1DM onset in NOD mice. This treatment increased Tregs proportions in the peripheral lymphocytes and influenced the bioactivity of spleen lymphocytes[99]. Beginning at 5 wk of age, oral administration of 10 µg of CTB-INS-GAD contained in silkworm pupae (every other day for 5 consecutive weeks) suppressed T1DM more effectively than insulin or GAD65 treatment alone in NOD mice. The synergistic beneficial effect of this combined dual antigen therapy was associated with restored Th1/Th2 imbalance by increasing Tregs proportions[100].

In addition to coupling T1DM autoantigens to CTB as described above, whether a nonconjugated form of CTB can improve oral tolerance induction was investigated. Mixing with CTB significantly enhanced the ability of orally administered insulin to induce tolerance. Admixtures of insulin and CTB at an optimal insulin:CTB ratio of 100:1 prevented diabetes in both LCMV induced mice and old NOD mice with established insulitis[101]. Apart from CTB, several other bacterial and plant enterotoxin B subunits were coupled to proinsulin or GAD. Beginning at 5 wk of age, oral feeding of 50 µg of these purified adjuvant-autoantigen proteins (once a week for 4 wk) reduced pancreatic insulitis levels significantly and induced a Th2 immune response[102].

As mentioned above, recombinant *L. lactis* has been applied to deliver T1DM autoantigens in combination with various immunomodulatory agents. Derived from human HSP60, P277 or DiaPep277 possesses potential roles to modulate immunological attack on β-cells in NOD mice[103], and serial clinical tests have been finished or are ongoing[104,105]. HSP65 from *Mycobacterium tuberculosis* may serve as an immunogenic vehicle for P277-based vaccines in NOD mice by nasal delivery of the fusion protein HSP65-6×P277[106]. This fusion protein was expressed in *L. lactis* and direct feeding of the recombinant strain (beginning at 4 wk of age and one time a day during the first week and then one time a week for 36 consecutive weeks) induced antigen-specific immunological tolerance and reduced the incidence of T1DM in NOD mice[84]. Based on the similar strategy, IA2P2, containing ten amino acids of B cell epitopes of autoantigen IA-2 and thirteen amino acids of human HSP60 (448-460), was expressed by *L. lactis* in form of HSP65-6×IA2P2. Direct feeding of the recombinant *L. lactis* prevented T1DM onset in NOD mice by formation of antigen-specific tolerance[86]. In addition, this research group developed the gut DCs-targeting NPs to deliver the above fusion protein HSP65-6×P277. The used NPs protected HSP65-6×P277 from degradation when passing through the GIT and significantly enhanced its uptake by DCs in the gut PPs. T1DM was prevented in all immunized mice by oral feeding of HSP65-6×P277-loaded targeting NPs and oral tolerance induction was achieved by repairing Th1/Th2 imbalance and increasing the functional CD4+Foxp3+CD25+ Tregs proportions[107].

The research group of Chantal Mathieu has also applied recombinant *L. lactis* to reverse diabetes in NOD mice. Combined with therapy with low-dose systemic anti-CD3 antibodies, oral feeding of *L. lactis* concurrently secreting human proinsulin and the immunomodulatory cytokine IL-10, which possesses the potential to improve the protective effect of oral insulin by mucosal administration[108], can stably reverse T1DM and autoantigen-specific long-term tolerance was induced in NOD mice[83]. Based on the similar strategy, a clinical-grade self-containing *L. lactis* formulation performed similar therapeutic efficacy in T1DM remission in NOD mice compared to the above plasmid-containing *L. lactis* strain and the therapy-induced tolerance depended on the emergence of functional Foxp3+ T cells[109]. Combined with short-course low-dose anti-CD3 antibodies, oral feeding of recombinant *L. lactis* secreting $GAD65_{370-575}$ and IL-10 can reverse diabetes in NOD mice by increasing the proportions of functional CD4+Foxp3+CD25+ Tregs[85]. Using preexisting anti-insulin antibodies as biomarkers, the research group of Matthias von Herrath showed that anti-CD3/oral insulin combination therapy induced long-term protection by increasing insulin-specific Tregs numbers in NOD mice[110]. As described above, anti-CD3 antibody has been used widely to modify the course of T1DM in animals or in clinical trials[111]. However, combination therapy with anti-CD6[112] or anti-CD20[113] and oral insulin failed to stimulate lasting tolerance and to prevent or reverse T1DM in mice.

In addition, compared to feeding insulin B-chain alone, coadministration of Schistosome egg antigens worked in a synergistic way to enhance Th2 type responses in NOD mice[68]. The immunoregulatory cytokine IL-4 and human GAD65 were expressed in transgenic tobacco plants, respectively. No protective effect was detected in NOD mice if either was fed alone, however, combined therapy by feeding plant-derived IL-4 and human GAD65 protected NOD mice from insulitis and T1DM by inducing oral tolerance[114].

Oral tolerance in clinical trials

Based on the effects obtained in animal models, clinical trials towards preventing or delaying T1DM in human by oral tolerance induction have been performed or are underway. Compared to oral tolerance trials in animals, in human clinical trials, insulin is the only used T1DM autoantigen. In 2000, there were two reports investigating whether tolerance can be induced in recent-onset T1DM individuals by oral delivery of insulin and both trials can be regarded as secondary prevention (Figure 2). Combined with intensive subcutaneous insulin treatment, oral administration of insulin (5 mg/d) was performed in patients with clinical T1DM (< 4 wk duration); however, this treatment made no sense to modify the disease course in the following year and no effect towards residual β-cell function was detected[115]. In the other report, oral insulin (2.5 or 7.5 mg/d) was administrated for 1 year and similarly, this treatment was useless to prevent deterioration in the function of residual β-cells in the first year after clinical diagnosis[116]. Therefore, the opinion that oral tolerance could be induced even when the immune process is initiated was challenged at that time and more experimental work may be required[117]. Two-dose (1 or 10 mg/d) oral insulin tolerance test in newly diagnosed (< 2 years) T1DM individuals was reported in 2004 and no clinical benefits were detected. However, a

chemical benefit was detected that 1 mg daily doses of oral insulin delayed progression of β-cell failure in patients diagnosed at ages above 20 years[118]. Whether oral tolerance can be induced by oral insulin was tested in the Diabetes Prevention Trial-Type 1 (DPT-1), which was designed as a secondary prevention trial (Figure 2). It showed that oral insulin (7.5 mg/d) had no effect in delaying or preventing T1DM in people who were autoantibody positive; however, the beneficial effect of delaying T1DM onset in patients with confirmed insulin autoantibody (IAA) levels ≥ 80 nU/mL was observed by subgroup analyses[119]. Longitudinal data analysis of IAA levels in the DPT-1 study showed that oral insulin (7.5 mg/d) did not affect IAA levels in individuals already present of IAA at onset of therapy[120]. The 13-year follow-up study indicated that the above beneficial effect observed in patients with IAA ≥ 80 nU/mL may be maintained as long as treatment continues[121]. These results suggested that IAA levels may be a key recruitment criterion for such studies. Promoted by the DPT-1 study, a randomized clinical trial (ClinicalTrials.gov Identifier: NCT00419562) showed that oral insulin (7.5 mg/d) exhibited no effect to delay or prevent T1DM over 2.7 years in autoantibody-positive relatives of patients with T1DM and oral insulin formulation applied by this study cannot be used for diabetes prevention[122]. As a primary prevention trial (Figure 2), the Pre-POINT Randomized Clinical Trial (ISRCTN76104595) fed T1DM-high-risk children who had no signs of islet autoimmunity with high doses of insulin and compared with placebo, oral insulin (67.5 mg/d) led to a regulatory immune response without hypoglycemia. The enhanced saliva IgG towards insulin and regulatory profiles of T-cells responding to insulin among treated individuals indicated the successful induction of oral tolerance[123]. Benefited from these promising findings observed in this small pilot study, a larger phase 2 trial (ClinicalTrials.gov Identifier: NCT02547519) is underway. In addition, there are some clinical trials that are underway, such as Frida insulin intervention (ClinicalTrials.gov Identifier: NCT02620072) titled "Mechanistic Study Using Oral Insulin for Immune Efficacy in Secondary Prevention of Type 1 Diabetes" containing two insulin dose strengths (7.5 mg/d and 67.5 mg/d) and oral insulin therapy for prevention of autoimmune diabetes in infants (4 mo to 7 mo) with high genetic risk: the GPPAD-POInT (Global Platform of Autoimmune Diabetes-Primary Oral Insulin Trial, ClinicalTrials.gov Identifier: NCT03364868)[124].

CONCLUSION

More detailed pathophysiological mechanisms responsible for the development of T1DM have been clarified by various studies performed during the past decades. However, the true cause of this disease remains unclear and translation of those above findings to an effective treatment has not been achieved. Working as an antigen-specific immunotherapy, oral tolerance therapy, if applied properly, exhibits the ability to restore the immune system towards durable tolerance to disease-related autoantigen(s). Various T cell subsets, such as Th1/2 and Tregs, and the related cytokines contribute to inducing oral tolerance, however, the potential signaling pathways controlling such immune responses contained in the development of oral tolerance are unclear and these processes as well as many aspects of the involved mechanisms need to be further studied to offer new targets for regulating tolerance.

As reviewed above, oral tolerance therapy for T1DM has been widely investigated in animal models, especially in NOD mice. Most previous studies indicate that oral administration of T1DM related autoantigens exhibits a positive effect to prevent, delay, or reverse the disease in NOD mice. However, these preclinical reports are somewhat conflicting in respect to the timing of therapy initiation, frequency and dosage of autoantigen administration, treatment duration, type of autoantigen applied, demand for combinational reagents and, perhaps most importantly, degree of efficacy. In addition, there are some reports indicating that oral autoantigen therapy exhibits no effect in delaying or preventing the onset of T1DM in animal models, possibly because antigens passing through the GIT are degraded, which leads to little autoantigen reaching the mucosal immune surface and subsequently influences tolerance induction. Therefore, a high amount of autoantigen may be needed and in order to circumvent this problem, some plant-, microbe-, or NPs-based delivery vehicles have been applied and shown promising outcomes. Based on the results obtained from oral autoantigen alone therapy, combinatorial therapy has been proposed by researchers to enhance tolerance induction. In addition to direct oral administration of different autoantigens (insulin and GAD65), various immune adjuvants, such as CTB and HSP65, and/or immunomodulatory agents, such as anti-

CD3 antibody, IL-10 and IL-4, have been applied in combination with oral administration of autoantigen(s). These combination therapies show synergy and perform potential as effective treatment options to enhance the protective effect of oral administration of autoantigen(s) by inducing tolerance in NOD mice.

Compared to trials applied in animals in which various autoantigens or combinational strategies have been performed, oral tolerance trials in human clinical trials mainly focus on the direct oral administration of various dosages of insulin. Although some oral insulin therapies for T1DM have been shown to be effective in animals, their clinical translation has not been achieved. Some important differences related to the progression of T1DM in animal models and humans may be responsible for this translation failure, such as the time course of disease, the speed and degree of destruction or recovery of β-cell, cytokine profile and function, male or female preference, and genetic polymorphisms. Furthermore, compared to the mouse models, humans exhibit more complicated regulation in the immune system, which may result in a lower immune response to the similar dosage of autoantigens. In addition, it should be noted that efficacy rather than safety is more addressed during the design and implementation of animal studies and the potential treatment-associated complications are less investigated or documented. However, the safety of oral tolerance induction should be considered when designing clinical trials and therefore, among the performed clinical trials, a lower dose of orally administered autoantigens is applied, and some combinational therapies using various delivery vehicles, immune adjuvants, or immunomodulatory agents have not been introduced.

As an antigen-based immunotherapy, we believe that oral tolerance therapy shows promise for restoring immune tolerance to autoantigen(s) and subsequently preventing, delaying, or reversing T1DM. To achieve this aim in human clinical trials, the following factors need to be carefully considered. First, immune biomarkers with improved sensitivity and specificity are needed to identify individuals at risk of T1DM as early as possible. Appropriate combination of immune biomarkers plays an absolutely important role in including the most appropriate target population into the right trials and evaluating treatment effects early. Second, since T1DM is a heterogeneous disease, all patients might not exhibit a similar response to a similar oral tolerance therapy. Therefore, more personalized strategies for tolerance induction may be needed to develop. Third, the results obtained from animal and human tests have shown that it may be far easier to prevent T1DM by oral tolerance therapy than to arrest or reverse its effects after clinical onset. Therefore, assuming that it is safe, oral tolerance therapy should be introduced in at-risk subjects, who are often children, as early as possible prior to the clinical onset of T1DM. Finally, animal studies have indicated that combinational therapies consisting of autoantigen administration with immune adjuvants and/or immunomodulatory agents possess the potential to safely prevent or stably reverse disease processes. Such combination studies in humans, which may be difficult to conduct, have not been published; however, these strategies still exhibit high therapeutic potential.

REFERENCES

1 **Expert Committee on the Diagnosis and Classification of Diabetes Mellitus**. Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care* 2003; **26** Suppl 1: S5-S20 [PMID: 12502614 DOI: 10.2337/diacare.26.2007.s5]

2 **DiMeglio LA**, Evans-Molina C, Oram RA. Type 1 diabetes. *Lancet* 2018; **391**: 2449-2462 [PMID: 29916386 DOI: 10.1016/S0140-6736(18)31320-5]

3 **Meetoo D**, McGovern P, Safadi R. An epidemiological overview of diabetes across the world. *Br J Nurs* 2007; **16**: 1002-1007 [PMID: 18026039 DOI: 10.12968/bjon.2007.16.16.27079]

4 **Maahs DM**, West NA, Lawrence JM, Mayer-Davis EJ. Epidemiology of type 1 diabetes. *Endocrinol Metab Clin North Am* 2010; **39**: 481-497 [PMID: 20723815 DOI: 10.1016/j.ecl.2010.05.011]

5 **Mayer-Davis EJ**, Dabelea D, Lawrence JM. Incidence Trends of Type 1 and Type 2 Diabetes among Youths, 2002-2012. *N Engl J Med* 2017; **377**: 301 [PMID: 28723318 DOI: 10.1056/NEJMc1706291]

6 **Chobot A**, Polanska J, Brandt A, Deja G, Glowinska-Olszewska B, Pilecki O, Szadkowska A, Mysliwiec M, Jarosz-Chobot P. Updated 24-year trend of Type 1 diabetes incidence in children in Poland reveals a sinusoidal pattern and sustained increase. *Diabet Med* 2017; **34**: 1252-1258 [PMID: 28257151 DOI: 10.1111/dme.13345]

7 **Thomas NJ**, Jones SE, Weedon MN, Shields BM, Oram RA, Hattersley AT. Frequency and phenotype of type 1 diabetes in the first six decades of life: a cross-sectional, genetically stratified survival analysis from UK Biobank. *Lancet Diabetes Endocrinol* 2018; **6**: 122-129 [PMID: 29199115 DOI: 10.1016/S2213-8587(17)30362-5]

8 **Roep BO**. The role of T-cells in the pathogenesis of Type 1 diabetes: from cause to cure. *Diabetologia* 2003; **46**: 305-321 [PMID: 12687328 DOI: 10.1007/s00125-003-1089-5]

9 **Panagiotopoulos C**, Trudeau JD, Tan R. T-cell epitopes in type 1 diabetes. *Curr Diab Rep* 2004; **4**: 87-94

[PMID: 15035967 DOI: 10.1007/s11892-004-0062-0]

10   **Krischer JP**, Lynch KF, Schatz DA, Ilonen J, Lernmark Å, Hagopian WA, Rewers MJ, She JX, Simell OG, Toppari J, Ziegler AG, Akolkar B, Bonifacio E; TEDDY Study Group. The 6 year incidence of diabetes-associated autoantibodies in genetically at-risk children: the TEDDY study. *Diabetologia* 2015; **58**: 980-987 [PMID: 25660258 DOI: 10.1007/s00125-015-3514-y]

11   **McLaughlin KA**, Richardson CC, Ravishankar A, Brigatti C, Liberati D, Lampasona V, Piemonti L, Morgan D, Feltbower RG, Christie MR. Identification of Tetraspanin-7 as a Target of Autoantibodies in Type 1 Diabetes. *Diabetes* 2016; **65**: 1690-1698 [PMID: 26953162 DOI: 10.2337/db15-1058]

12   **Rich SS**, Akolkar B, Concannon P, Erlich H, Hilner JE, Julier C, Morahan G, Nerup J, Nierras C, Pociot F, Todd JA. Overview of the Type I Diabetes Genetics Consortium. *Genes Immun* 2009; **10** Suppl 1: S1-S4 [PMID: 19956093 DOI: 10.1038/gene.2009.84]

13   **Robertson CC**, Rich SS. Genetics of type 1 diabetes. *Curr Opin Genet Dev* 2018; **50**: 7-16 [PMID: 29453110 DOI: 10.1016/j.gde.2018.01.006]

14   **Redondo MJ**, Steck AK, Pugliese A. Genetics of type 1 diabetes. *Pediatr Diabetes* 2018; **19**: 346-353 [PMID: 29094512 DOI: 10.1111/pedi.12597]

15   **TEDDY Study Group**. The Environmental Determinants of Diabetes in the Young (TEDDY) study: study design. *Pediatr Diabetes* 2007; **8**: 286-298 [PMID: 17850472 DOI: 10.1111/j.1399-5448.2007.00269.x]

16   **Regnell SE**, Lernmark Å. Early prediction of autoimmune (type 1) diabetes. *Diabetologia* 2017; **60**: 1370-1381 [PMID: 28550517 DOI: 10.1007/s00125-017-4308-1]

17   **Oram RA**, Patel K, Hill A, Shields B, McDonald TJ, Jones A, Hattersley AT, Weedon MN. A Type 1 Diabetes Genetic Risk Score Can Aid Discrimination Between Type 1 and Type 2 Diabetes in Young Adults. *Diabetes Care* 2016; **39**: 337-344 [PMID: 26577414 DOI: 10.2337/dc15-1111]

18   **Winkler C**, Krumsiek J, Buettner F, Angermüller C, Giannopoulou EZ, Theis FJ, Ziegler AG, Bonifacio E. Feature ranking of type 1 diabetes susceptibility genes improves prediction of type 1 diabetes. *Diabetologia* 2014; **57**: 2521-2529 [PMID: 25186292 DOI: 10.1007/s00125-014-3362-1]

19   **Atkinson MA**, Eisenbarth GS, Michels AW. Type 1 diabetes. *Lancet* 2014; **383**: 69-82 [PMID: 23890997 DOI: 10.1016/S0140-6736(13)60591-7]

20   **Achenbach P**, Warncke K, Reiter J, Naserke HE, Williams AJ, Bingley PJ, Bonifacio E, Ziegler AG. Stratification of type 1 diabetes risk on the basis of islet autoantibody characteristics. *Diabetes* 2004; **53**: 384-392 [PMID: 14747289 DOI: 10.2337/diabetes.53.2.384]

21   **Vehik K**, Beam CA, Mahon JL, Schatz DA, Haller MJ, Sosenko JM, Skyler JS, Krischer JP; TrialNet Natural History Study Group. Development of autoantibodies in the TrialNet Natural History Study. *Diabetes Care* 2011; **34**: 1897-1901 [PMID: 21750277 DOI: 10.2337/dc11-0560]

22   **Ziegler AG**, Rewers M, Simell O, Simell T, Lempainen J, Steck A, Winkler C, Ilonen J, Veijola R, Knip M, Bonifacio E, Eisenbarth GS. Seroconversion to multiple islet autoantibodies and risk of progression to diabetes in children. *JAMA* 2013; **309**: 2473-2479 [PMID: 23780460 DOI: 10.1001/jama.2013.6285]

23   **Insel RA**, Dunne JL, Atkinson MA, Chiang JL, Dabelea D, Gottlieb PA, Greenbaum CJ, Herold KC, Krischer JP, Lernmark Å, Ratner RE, Rewers MJ, Schatz DA, Skyler JS, Sosenko JM, Ziegler AG. Staging presymptomatic type 1 diabetes: a scientific statement of JDRF, the Endocrine Society, and the American Diabetes Association. *Diabetes Care* 2015; **38**: 1964-1974 [PMID: 26404926 DOI: 10.2337/dc15-1419]

24   **Dayan CM**, Korah M, Tatovic D, Bundy BN, Herold KC. Changing the landscape for type 1 diabetes: the first step to prevention. *Lancet* 2019; **394**: 1286-1296 [PMID: 31533907 DOI: 10.1016/S0140-6736(19)32127-0]

25   **Coppieters KT**, Harrison LC, von Herrath MG. Trials in type 1 diabetes: Antigen-specific therapies. *Clin Immunol* 2013; **149**: 345-355 [PMID: 23490422 DOI: 10.1016/j.clim.2013.02.002]

26   **Simmons KM**, Michels AW. Type 1 diabetes: A predictable disease. *World J Diabetes* 2015; **6**: 380-390 [PMID: 25897349 DOI: 10.4239/wjd.v6.i3.380]

27   **Ilonen J**, Lempainen J, Veijola R. The heterogeneous pathogenesis of type 1 diabetes mellitus. *Nat Rev Endocrinol* 2019; **15**: 635-650 [PMID: 31534209 DOI: 10.1038/s41574-019-0254-y]

28   **Staeva-Vieira T**, Peakman M, von Herrath M. Translational mini-review series on type 1 diabetes: Immune-based therapeutic approaches for type 1 diabetes. *Clin Exp Immunol* 2007; **148**: 17-31 [PMID: 17349010 DOI: 10.1111/j.1365-2249.2007.03328.x]

29   **Jacobsen LM**, Newby BN, Perry DJ, Posgai AL, Haller MJ, Brusko TM. Immune Mechanisms and Pathways Targeted in Type 1 Diabetes. *Curr Diab Rep* 2018; **18**: 90 [PMID: 30168021 DOI: 10.1007/s11892-018-1066-5]

30   **Rezende RM**, Weiner HL. History and mechanisms of oral tolerance. *Semin Immunol* 2017; **30**: 3-11 [PMID: 28774470 DOI: 10.1016/j.smim.2017.07.004]

31   **Wambre E**, Jeong D. Oral Tolerance Development and Maintenance. *Immunol Allergy Clin North Am* 2018; **38**: 27-37 [PMID: 29132672 DOI: 10.1016/j.iac.2017.09.003]

32   **Knoop KA**, Miller MJ, Newberry RD. Transepithelial antigen delivery in the small intestine: different paths, different outcomes. *Curr Opin Gastroenterol* 2013; **29**: 112-118 [PMID: 23380572 DOI: 10.1097/MOG.0b013e32835cf1cd]

33   **Tordesillas L**, Berin MC. Mechanisms of Oral Tolerance. *Clin Rev Allergy Immunol* 2018; **55**: 107-117 [PMID: 29488131 DOI: 10.1007/s12016-018-8680-5]

34   **Yoshida M**, Claypool SM, Wagner JS, Mizoguchi E, Mizoguchi A, Roopenian DC, Lencer WI, Blumberg RS. Human neonatal Fc receptor mediates transport of IgG into luminal secretions for delivery of antigens to mucosal dendritic cells. *Immunity* 2004; **20**: 769-783 [PMID: 15189741 DOI: 10.1016/j.immuni.2004.05.007]

35   **Niess JH**, Brand S, Gu X, Landsman L, Jung S, McCormick BA, Vyas JM, Boes M, Ploegh HL, Fox JG, Littman DR, Reinecker HC. CX3CR1-mediated dendritic cell access to the intestinal lumen and bacterial clearance. *Science* 2005; **307**: 254-258 [PMID: 15653504 DOI: 10.1126/science.1102901]

36   **Mazzini E**, Massimiliano L, Penna G, Rescigno M. Oral tolerance can be established *via* gap junction transfer of fed antigens from CX3CR1^{+} macrophages to CD103^{+} dendritic cells. *Immunity* 2014; **40**: 248-

261 [PMID: 24462723 DOI: 10.1016/j.immuni.2013.12.012]

37 **Satitsuksanoa P**, Jansen K, Głobińska A, van de Veen W, Akdis M. Regulatory Immune Mechanisms in Tolerance to Food Allergy. *Front Immunol* 2018; **9**: 2939 [PMID: 30619299 DOI: 10.3389/fimmu.2018.02939]

38 **McDole JR**, Wheeler LW, McDonald KG, Wang B, Konjufca V, Knoop KA, Newberry RD, Miller MJ. Goblet cells deliver luminal antigen to CD103+ dendritic cells in the small intestine. *Nature* 2012; **483**: 345-349 [PMID: 22422267 DOI: 10.1038/nature10863]

39 **Mowat AM**. To respond or not to respond-a personal perspective of intestinal tolerance. *Nat Rev Immunol* 2018; **18**: 405-415 [PMID: 29491358 DOI: 10.1038/s41577-018-0002-x]

40 **Jang MH**, Kweon MN, Iwatani K, Yamamoto M, Terahara K, Sasakawa C, Suzuki T, Nochi T, Yokota Y, Rennert PD, Hiroi T, Tamagawa H, Iijima H, Kunisawa J, Yuki Y, Kiyono H. Intestinal villous M cells: an antigen entry site in the mucosal epithelium. *Proc Natl Acad Sci USA* 2004; **101**: 6110-6115 [PMID: 15071180 DOI: 10.1073/pnas.0400969101]

41 **Schulz O**, Jaensson E, Persson EK, Liu X, Worbs T, Agace WW, Pabst O. Intestinal CD103+, but not CX3CR1+, antigen sampling cells migrate in lymph and serve classical dendritic cell functions. *J Exp Med* 2009; **206**: 3101-3114 [PMID: 20008524 DOI: 10.1084/jem.20091925]

42 **Worbs T**, Bode U, Yan S, Hoffmann MW, Hintzen G, Bernhardt G, Förster R, Pabst O. Oral tolerance originates in the intestinal immune system and relies on antigen carriage by dendritic cells. *J Exp Med* 2006; **203**: 519-527 [PMID: 16533884 DOI: 10.1084/jem.20052016]

43 **Spahn TW**, Weiner HL, Rennert PD, Lügering N, Fontana A, Domschke W, Kucharzik T. Mesenteric lymph nodes are critical for the induction of high-dose oral tolerance in the absence of Peyer's patches. *Eur J Immunol* 2002; **32**: 1109-1113 [PMID: 11920578 DOI: 10.1002/1521-4141(200204)32:4<1109::AID-IMMU1109>3.0.CO;2-K]

44 **Kraus TA**, Brimnes J, Muong C, Liu JH, Moran TM, Tappenden KA, Boros P, Mayer L. Induction of mucosal tolerance in Peyer's patch-deficient, ligated small bowel loops. *J Clin Invest* 2005; **115**: 2234-2243 [PMID: 16041410 DOI: 10.1172/JCI19102]

45 **Sricharunrat T**, Pumirat P, Leaungwutiwong P. Oral tolerance:Recent advances on mechanisms and potential applications. *Asian Pac J Allergy Immunol* 2018; **36**: 207-216 [PMID: 30599095 DOI: 10.12932/AP0848]

46 **Cassani B**, Villablanca EJ, Quintana FJ, Love PE, Lacy-Hulbert A, Blaner WS, Sparwasser T, Snapper SB, Weiner HL, Mora JR. Gut-tropic T cells that express integrin α4β7 and CCR9 are required for induction of oral immune tolerance in mice. *Gastroenterology* 2011; **141**: 2109-2118 [PMID: 21925467 DOI: 10.1053/j.gastro.2011.09.015]

47 **Hadis U**, Wahl B, Schulz O, Hardtke-Wolenski M, Schippers A, Wagner N, Müller W, Sparwasser T, Förster R, Pabst O. Intestinal tolerance requires gut homing and expansion of FoxP3+ regulatory T cells in the lamina propria. *Immunity* 2011; **34**: 237-246 [PMID: 21333554 DOI: 10.1016/j.immuni.2011.01.016]

48 **Gregori S**, Passerini L, Roncarolo MG. Clinical Outlook for Type-1 and FOXP3(+) T Regulatory Cell-Based Therapy. *Front Immunol* 2015; **6**: 593 [PMID: 26635807 DOI: 10.3389/fimmu.2015.00593]

49 **Weiner HL**. Oral tolerance: immune mechanisms and the generation of Th3-type TGF-beta-secreting regulatory cells. *Microbes Infect* 2001; **3**: 947-954 [PMID: 11564443 DOI: 10.1016/s1286-4579(01)01456-3]

50 **Chen Y**, Inobe J, Kuchroo VK, Baron JL, Janeway CA Jr, Weiner HL. Oral tolerance in myelin basic protein T-cell receptor transgenic mice: suppression of autoimmune encephalomyelitis and dose-dependent induction of regulatory cells. *Proc Natl Acad Sci USA* 1996; **93**: 388-391 [PMID: 8552644 DOI: 10.1073/pnas.93.1.388]

51 **Dubois B**, Chapat L, Goubier A, Papiernik M, Nicolas JF, Kaiserlian D. Innate CD4+CD25+ regulatory T cells are required for oral tolerance and inhibition of CD8+ T cells mediating skin inflammation. *Blood* 2003; **102**: 3295-3301 [PMID: 12855551 DOI: 10.1182/blood-2003-03-0727]

52 **Pabst O**, Mowat AM. Oral tolerance to food protein. *Mucosal Immunol* 2012; **5**: 232-239 [PMID: 22318493 DOI: 10.1038/mi.2012.4]

53 **Thomson AW**, Knolle PA. Antigen-presenting cell function in the tolerogenic liver environment. *Nat Rev Immunol* 2010; **10**: 753-766 [PMID: 20972472 DOI: 10.1038/nri2858]

54 **Goubier A**, Dubois B, Gheit H, Joubert G, Villard-Truc F, Asselin-Paturel C, Trinchieri G, Kaiserlian D. Plasmacytoid dendritic cells mediate oral tolerance. *Immunity* 2008; **29**: 464-475 [PMID: 18789731 DOI: 10.1016/j.immuni.2008.06.017]

55 **Mascarell L**, Lombardi V, Louise A, Saint-Lu N, Chabre H, Moussu H, Betbeder D, Balazuc AM, Van Overtvelt L, Moingeon P. Oral dendritic cells mediate antigen-specific tolerance by stimulating TH1 and regulatory CD4+ T cells. *J Allergy Clin Immunol* 2008; **122**: 603-9.e5 [PMID: 18774396 DOI: 10.1016/j.jaci.2008.06.034]

56 **Palomares O**, Rückert B, Jartti T, Kücüksezer UC, Puhakka T, Gomez E, Fahrner HB, Speiser A, Jung A, Kwok WW, Kalogjera L, Akdis M, Akdis CA. Induction and maintenance of allergen-specific FOXP3+ Treg cells in human tonsils as potential first-line organs of oral tolerance. *J Allergy Clin Immunol* 2012; **129**: 510-520, 520.e1 [PMID: 22051696 DOI: 10.1016/j.jaci.2011.09.031]

57 **Burton OT**, Tamayo JM, Stranks AJ, Koleoglou KJ, Oettgen HC. Allergen-specific IgG antibody signaling through FcγRIIb promotes food tolerance. *J Allergy Clin Immunol* 2018; **141**: 189-201.e3 [PMID: 28479335 DOI: 10.1016/j.jaci.2017.03.045]

58 **Du Toit G**, Roberts G, Sayre PH, Bahnson HT, Radulovic S, Santos AF, Brough HA, Phippard D, Basting M, Feeney M, Turcanu V, Sever ML, Gomez Lorenzo M, Plaut M, Lack G; LEAP Study Team. Randomized trial of peanut consumption in infants at risk for peanut allergy. *N Engl J Med* 2015; **372**: 803-813 [PMID: 25705822 DOI: 10.1056/NEJMoa1414850]

59 **Badenhoop K**, Kahles H, Penna-Martinez M. Vitamin D, immune tolerance, and prevention of type 1 diabetes. *Curr Diab Rep* 2012; **12**: 635-642 [PMID: 22976537 DOI: 10.1007/s11892-012-0322-3]

60 **Harrison LC**, Wentworth JM, Zhang Y, Bandala-Sanchez E, Böhmer RM, Neale AM, Stone NL, Naselli G, Bosco JJ, Auyeung P, Rashidi M, Augstein P, Morahan G. Antigen-based vaccination and prevention of

type 1 diabetes. *Curr Diab Rep* 2013; **13**: 616-623 [PMID: 23888323 DOI: 10.1007/s11892-013-0415-7]

61   **Nicholas D**, Odumosu O, Langridge WH. Autoantigen based vaccines for type 1 diabetes. *Discov Med* 2011; **11**: 293-301 [PMID: 21524383]

62   **Zhang ZJ**, Davidson L, Eisenbarth G, Weiner HL. Suppression of diabetes in nonobese diabetic mice by oral administration of porcine insulin. *Proc Natl Acad Sci USA* 1991; **88**: 10252-10256 [PMID: 1946445 DOI: 10.1073/pnas.88.22.10252]

63   **Makino S**, Kunimoto K, Muraoka Y, Mizushima Y, Katagiri K, Tochino Y. Breeding of a non-obese, diabetic strain of mice. *Jikken Dobutsu* 1980; **29**: 1-13 [PMID: 6995140 DOI: 10.1538/expanim1978.29.1_1]

64   **Weiner HL**. Current issues in the treatment of human diseases by mucosal tolerance. *Ann N Y Acad Sci* 2004; **1029**: 211-224 [PMID: 15681760 DOI: 10.1196/annals.1309.053]

65   **Maron R**, Blogg NS, Polanski M, Hancock W, Weiner HL. Oral tolerance to insulin and the insulin B-chain: cell lines and cytokine patterns. *Ann N Y Acad Sci* 1996; **778**: 346-357 [PMID: 8610988 DOI: 10.1111/j.1749-6632.1996.tb21142.x]

66   **Hancock WW**, Polanski M, Zhang J, Blogg N, Weiner HL. Suppression of insulitis in non-obese diabetic (NOD) mice by oral insulin administration is associated with selective expression of interleukin-4 and -10, transforming growth factor-beta, and prostaglandin-E. *Am J Pathol* 1995; **147**: 1193-1199 [PMID: 7485382]

67   **Polanski M**, Melican NS, Zhang J, Weiner HL. Oral administration of the immunodominant B-chain of insulin reduces diabetes in a co-transfer model of diabetes in the NOD mouse and is associated with a switch from Th1 to Th2 cytokines. *J Autoimmun* 1997; **10**: 339-346 [PMID: 9237797 DOI: 10.1006/jaut.1997.0148]

68   **Maron R**, Palanivel V, Weiner HL, Harn DA. Oral administration of schistosome egg antigens and insulin B-chain generates and enhances Th2-type responses in NOD mice. *Clin Immunol Immunopathol* 1998; **87**: 85-92 [PMID: 9576014 DOI: 10.1006/clin.1997.4506]

69   **Maron R**, Guerau-de-Arellano M, Zhang X, Weiner HL. Oral administration of insulin to neonates suppresses spontaneous and cyclophosphamide induced diabetes in the NOD mouse. *J Autoimmun* 2001; **16**: 21-28 [PMID: 11221993 DOI: 10.1006/jaut.2000.0471]

70   **Maron R**, Melican NS, Weiner HL. Regulatory Th2-type T cell lines against insulin and GAD peptides derived from orally- and nasally-treated NOD mice suppress diabetes. *J Autoimmun* 1999; **12**: 251-258 [PMID: 10330296 DOI: 10.1006/jaut.1999.0278]

71   **Bergerot I**, Fabien N, Maguer V, Thivolet C. Oral administration of human insulin to NOD mice generates CD4+ T cells that suppress adoptive transfer of diabetes. *J Autoimmun* 1994; **7**: 655-663 [PMID: 7840857 DOI: 10.1006/jaut.1994.1050]

72   **Ploix C**, Bergerot I, Fabien N, Perche S, Moulin V, Thivolet C. Protection against autoimmune diabetes with oral insulin is associated with the presence of IL-4 type 2 T-cells in the pancreas and pancreatic lymph nodes. *Diabetes* 1998; **47**: 39-44 [PMID: 9421372 DOI: 10.2337/diab.47.1.39]

73   **Bergerot I**, Arreaza GA, Cameron MJ, Burdick MD, Strieter RM, Chensue SW, Chakrabarti S, Delovitch TL. Insulin B-chain reactive CD4+ regulatory T-cells induced by oral insulin treatment protect from type 1 diabetes by blocking the cytokine secretion and pancreatic infiltration of diabetogenic effector T-cells. *Diabetes* 1999; **48**: 1720-1729 [PMID: 10480600 DOI: 10.2337/diabetes.48.9.1720]

74   **von Herrath MG**, Dyrberg T, Oldstone MB. Oral insulin treatment suppresses virus-induced antigen-specific destruction of beta cells and prevents autoimmune diabetes in transgenic mice. *J Clin Invest* 1996; **98**: 1324-1331 [PMID: 8823297 DOI: 10.1172/JCI118919]

75   **Homann D**, Dyrberg T, Petersen J, Oldstone MB, von Herrath MG. Insulin in oral immune "tolerance": a one-amino acid change in the B chain makes the difference. *J Immunol* 1999; **163**: 1833-1838 [PMID: 10438916]

76   **Mordes JP**, Schirf B, Roipko D, Greiner DL, Weiner H, Nelson P, Rossini AA. Oral insulin does not prevent insulin-dependent diabetes mellitus in BB rats. *Ann N Y Acad Sci* 1996; **778**: 418-421 [PMID: 8611008 DOI: 10.1111/j.1749-6632.1996.tb21161.x]

77   **Ramiya VK**, Shang XZ, Wasserfall CH, Maclaren NK. Effect of oral and intravenous insulin and glutamic acid decarboxylase in NOD mice. *Autoimmunity* 1997; **26**: 139-151 [PMID: 9550282 DOI: 10.3109/08916939708994736]

78   **Pham MN**, Gibson C, Rydén AK, Perdue N, Boursalian TE, Pagni PP, Coppieters K, Skonberg C, Porsgaard T, von Herrath M, Vela JL. Oral insulin (human, murine, or porcine) does not prevent diabetes in the non-obese diabetic mouse. *Clin Immunol* 2016; **164**: 28-33 [PMID: 26821303 DOI: 10.1016/j.clim.2016.01.013]

79   **Ma SW**, Zhao DL, Yin ZQ, Mukherjee R, Singh B, Qin HY, Stiller CR, Jevnikar AM. Transgenic plants expressing autoantigens fed to mice to induce oral immune tolerance. *Nat Med* 1997; **3**: 793-796 [PMID: 9212110 DOI: 10.1038/nm0797-793]

80   **Posgai AL**, Wasserfall CH, Kwon KC, Daniell H, Schatz DA, Atkinson MA. Plant-based vaccines for oral delivery of type 1 diabetes-related autoantigens: Evaluating oral tolerance mechanisms and disease prevention in NOD mice. *Sci Rep* 2017; **7**: 42372 [PMID: 28205558 DOI: 10.1038/srep42372]

81   **Daniel C**, Roussel Y, Kleerebezem M, Pot B. Recombinant lactic acid bacteria as mucosal biotherapeutic agents. *Trends Biotechnol* 2011; **29**: 499-508 [PMID: 21665301 DOI: 10.1016/j.tibtech.2011.05.002]

82   **Cook DP**, Gysemans C, Mathieu C. *Lactococcus lactis* As a Versatile Vehicle for Tolerogenic Immunotherapy. *Front Immunol* 2017; **8**: 1961 [PMID: 29387056 DOI: 10.3389/fimmu.2017.01961]

83   **Takiishi T**, Korf H, Van Belle TL, Robert S, Grieco FA, Caluwaerts S, Galleri L, Spagnuolo I, Steidler L, Van Huynegem K, Demetter P, Wasserfall C, Atkinson MA, Dotta F, Rottiers P, Gysemans C, Mathieu C. Reversal of autoimmune diabetes by restoration of antigen-specific tolerance using genetically modified Lactococcus lactis in mice. *J Clin Invest* 2012; **122**: 1717-1725 [PMID: 22484814 DOI: 10.1172/JCI60530]

84   **Ma Y**, Liu J, Hou J, Dong Y, Lu Y, Jin L, Cao R, Li T, Wu J. Oral administration of recombinant Lactococcus lactis expressing HSP65 and tandemly repeated P277 reduces the incidence of type I diabetes

in non-obese diabetic mice. *PLoS One* 2014; **9**: e105701 [PMID: 25157497 DOI: 10.1371/journal.pone.0105701]

85   **Robert S**, Gysemans C, Takiishi T, Korf H, Spagnuolo I, Sebastiani G, Van Huynegem K, Steidler L, Caluwaerts S, Demetter P, Wasserfall CH, Atkinson MA, Dotta F, Rottiers P, Van Belle TL, Mathieu C. Oral delivery of glutamic acid decarboxylase (GAD)-65 and IL10 by Lactococcus lactis reverses diabetes in recent-onset NOD mice. *Diabetes* 2014; **63**: 2876-2887 [PMID: 24677716 DOI: 10.2337/db13-1236]

86   **Liu KF**, Liu XR, Li GL, Lu SP, Jin L, Wu J. Oral administration of Lactococcus lactis-expressing heat shock protein 65 and tandemly repeated IA2P2 prevents type 1 diabetes in NOD mice. *Immunol Lett* 2016; **174**: 28-36 [PMID: 27085380 DOI: 10.1016/j.imlet.2016.04.008]

87   **Mao R**, Chen Y, Chi Z, Wang Y. Insulin and its single-chain analogue. *Appl Microbiol Biotechnol* 2019; **103**: 8737-8751 [PMID: 31637493 DOI: 10.1007/s00253-019-10170-0]

88   **Ng DT**, Sarkar CA. Nisin-inducible secretion of a biologically active single-chain insulin analog by Lactococcus lactis NZ9000. *Biotechnol Bioeng* 2011; **108**: 1987-1996 [PMID: 21391210 DOI: 10.1002/bit.23130]

89   **Mao R**, Wu D, Hu S, Zhou K, Wang M, Wang Y. Secretory expression and surface display of a new and biologically active single-chain insulin (SCI-59) analog by lactic acid bacteria. *Appl Microbiol Biotechnol* 2017; **101**: 3259-3271 [PMID: 28120013 DOI: 10.1007/s00253-017-8125-8]

90   **Mao R**, Wu D, Wang Y. Surface display on lactic acid bacteria without genetic modification: strategies and applications. *Appl Microbiol Biotechnol* 2016; **100**: 9407-9421 [PMID: 27649963 DOI: 10.1007/s00253-016-7842-8]

91   **Mao R**, Chen Y, Wu Q, Zhang T, Diao E, Wu D, Wang M, Liu Y, Lu L, Chang X, Zheng Y, Wang Y. Oral delivery of single-chain insulin (SCI-59) analog by bacterium-like particles (BLPs) induces oral tolerance and prevents autoimmune diabetes in NOD mice. *Immunol Lett* 2019; **214**: 37-44 [PMID: 31473255 DOI: 10.1016/j.imlet.2019.08.008]

92   **Gong Z**, Jin Y, Zhang Y. Oral administration of a cholera toxin B subunit-insulin fusion protein produced in silkworm protects against autoimmune diabetes. *J Biotechnol* 2005; **119**: 93-105 [PMID: 16024127 DOI: 10.1016/j.jbiotec.2005.05.027]

93   **Arakawa T**, Yu J, Chong DK, Hough J, Engen PC, Langridge WH. A plant-based cholera toxin B subunit-insulin fusion protein protects against the development of autoimmune diabetes. *Nat Biotechnol* 1998; **16**: 934-938 [PMID: 9788349 DOI: 10.1038/nbt1098-934]

94   **Bergerot I**, Ploix C, Petersen J, Moulin V, Rask C, Fabien N, Lindblad M, Mayer A, Czerkinsky C, Holmgren J, Thivolet C. A cholera toxoid-insulin conjugate as an oral vaccine against spontaneous autoimmune diabetes. *Proc Natl Acad Sci USA* 1997; **94**: 4610-4614 [PMID: 9114038 DOI: 10.1073/pnas.94.9.4610]

95   **Ploix C**, Bergerot I, Durand A, Czerkinsky C, Holmgren J, Thivolet C. Oral administration of cholera toxin B-insulin conjugates protects NOD mice from autoimmune diabetes by inducing CD4+ regulatory T-cells. *Diabetes* 1999; **48**: 2150-2156 [PMID: 10535448 DOI: 10.2337/diabetes.48.11.2150]

96   **Petersen JS**, Bregenholt S, Apostolopolous V, Homann D, Wolfe T, Hughes A, De Jongh K, Wang M, Dyrberg T, Von Herrath MG. Coupling of oral human or porcine insulin to the B subunit of cholera toxin (CTB) overcomes critical antigenic differences for prevention of type I diabetes. *Clin Exp Immunol* 2003; **134**: 38-45 [PMID: 12974752 DOI: 10.1046/j.1365-2249.2003.02255.x]

97   **Ruhlman T**, Ahangari R, Devine A, Samsam M, Daniell H. Expression of cholera toxin B-proinsulin fusion protein in lettuce and tobacco chloroplasts--oral administration protects against development of insulitis in non-obese diabetic mice. *Plant Biotechnol J* 2007; **5**: 495-510 [PMID: 17490448 DOI: 10.1111/j.1467-7652.2007.00259.x]

98   **Gong Z**, Jin Y, Zhang Y. Suppression of diabetes in non-obese diabetic (NOD) mice by oral administration of a cholera toxin B subunit-insulin B chain fusion protein vaccine produced in silkworm. *Vaccine* 2007; **25**: 1444-1451 [PMID: 17113687 DOI: 10.1016/j.vaccine.2006.10.039]

99   **Meng Q**, Wang W, Shi X, Jin Y, Zhang Y. Protection against autoimmune diabetes by silkworm-produced GFP-tagged CTB-insulin fusion protein. *Clin Dev Immunol* 2011; **2011**: 831704 [PMID: 21765853 DOI: 10.1155/2011/831704]

100   **Liu B**, Yue Y, Yang Y, Jin Y. Oral Administration of Silkworm-Produced GAD65 and Insulin Bi-Autoantigens against Type 1 Diabetes. *PLoS One* 2016; **11**: e0147260 [PMID: 26783749 DOI: 10.1371/journal.pone.0147260]

101   **Bregenholt S**, Wang M, Wolfe T, Hughes A, Baerentzen L, Dyrberg T, von Herrath MG, Petersen JS. The cholera toxin B subunit is a mucosal adjuvant for oral tolerance induction in type 1 diabetes. *Scand J Immunol* 2003; **57**: 432-438 [PMID: 12753499 DOI: 10.1046/j.1365-3083.2003.01248.x]

102   **Carter JE 3rd**, Yu J, Choi NW, Hough J, Henderson D, He D, Langridge WH. Bacterial and plant enterotoxin B subunit-autoantigen fusion proteins suppress diabetes insulitis. *Mol Biotechnol* 2006; **32**: 1-15 [PMID: 16382177 DOI: 10.1385/MB:32:1:001]

103   **Elias D**, Cohen IR. Peptide therapy for diabetes in NOD mice. *Lancet* 1994; **343**: 704-706 [PMID: 7907681 DOI: 10.1016/s0140-6736(94)91582-2]

104   **Cook DP**, Gysemans C, Mathieu C. Prospects of a type 1 diabetes vaccine. *Expert Opin Biol Ther* 2017; **17**: 403-406 [PMID: 28274141 DOI: 10.1080/14712598.2017.1295035]

105   **Robert S**, Korf H, Gysemans C, Mathieu C. Antigen-based vs. *Islets* 2013; **5**: 53-66 [PMID: 23648893 DOI: 10.4161/isl.24785]

106   **Liang J**, Aihua Z, Yu W, Yong L, Jingjing L. HSP65 serves as an immunogenic carrier for a diabetogenic peptide P277 inducing anti-inflammatory immune response in NOD mice by nasal administration. *Vaccine* 2010; **28**: 3312-3317 [PMID: 20226247 DOI: 10.1016/j.vaccine.2010.02.100]

107   **Chen Y**, Wu J, Wang J, Zhang W, Xu B, Xu X, Zong L. Targeted delivery of antigen to intestinal dendritic cells induces oral tolerance and prevents autoimmune diabetes in NOD mice. *Diabetologia* 2018; **61**: 1384-1396 [PMID: 29546475 DOI: 10.1007/s00125-018-4593-3]

108   **Slavin AJ**, Maron R, Weiner HL. Mucosal administration of IL-10 enhances oral tolerance in autoimmune encephalomyelitis and diabetes. *Int Immunol* 2001; **13**: 825-833 [PMID: 11369711 DOI:

10.1093/intimm/13.6.825]

109   **Takiishi T**, Cook DP, Korf H, Sebastiani G, Mancarella F, Cunha JP, Wasserfall C, Casares N, Lasarte JJ, Steidler L, Rottiers P, Dotta F, Gysemans C, Mathieu C. Reversal of Diabetes in NOD Mice by Clinical-Grade Proinsulin and IL-10-Secreting Lactococcus lactis in Combination With Low-Dose Anti-CD3 Depends on the Induction of Foxp3-Positive T Cells. *Diabetes* 2017; **66**: 448-459 [PMID: 28108611 DOI: 10.2337/db15-1625]

110   **Mamchak AA**, Manenkova Y, Leconet W, Zheng Y, Chan JR, Stokes CL, Shoda LK, von Herrath M, Bresson D. Preexisting autoantibodies predict efficacy of oral insulin to cure autoimmune diabetes in combination with anti-CD3. *Diabetes* 2012; **61**: 1490-1499 [PMID: 22362174 DOI: 10.2337/db11-1304]

111   **Gaglia J**, Kissler S. Anti-CD3 Antibody for the Prevention of Type 1 Diabetes: A Story of Perseverance. *Biochemistry* 2019; **58**: 4107-4111 [PMID: 31523950 DOI: 10.1021/acs.biochem.9b00707]

112   **Schneider DA**, Sarikonda G, Montero E, von Herrath MG. Combination therapy with anti-CD6 and oral insulin immunization reverses recent onset diabetes in non obese diabetic mice but fails to induce lasting tolerance. *Clin Immunol* 2013; **149**: 440-441 [PMID: 24211845 DOI: 10.1016/j.clim.2013.08.004]

113   **Sarikonda G**, Sachithanantham S, Manenkova Y, Kupfer T, Posgai A, Wasserfall C, Bernstein P, Straub L, Pagni PP, Schneider D, Calvo TR, Coulombe M, Herold K, Gill RG, Atkinson M, Nepom G, Ehlers M, Staeva T, Garren H, Steinman L, Chan AC, von Herrath M. Transient B-cell depletion with anti-CD20 in combination with proinsulin DNA vaccine or oral insulin: immunologic effects and efficacy in NOD mice. *PLoS One* 2013; **8**: e54712 [PMID: 23405091 DOI: 10.1371/journal.pone.0054712]

114   **Ma S**, Huang Y, Yin Z, Menassa R, Brandle JE, Jevnikar AM. Induction of oral tolerance to prevent diabetes with transgenic plants requires glutamic acid decarboxylase (GAD) and IL-4. *Proc Natl Acad Sci USA* 2004; **101**: 5680-5685 [PMID: 15056761 DOI: 10.1073/pnas.0307420101]

115   **Pozzilli P**, Pitocco D, Visalli N, Cavallo MG, Buzzetti R, Crinò A, Spera S, Suraci C, Multari G, Cervoni M, Manca Bitti ML, Matteoli MC, Marietti G, Ferrazzoli F, Cassone Faldetta MR, Giordano C, Sbriglia M, Sarugeri E, Ghirlanda G. No effect of oral insulin on residual beta-cell function in recent-onset type I diabetes (the IMDIAB VII). *Diabetologia* 2000; **43**: 1000-1004 [PMID: 10990077 DOI: 10.1007/s001250051482]

116   **Chaillous L**, Lefèvre H, Thivolet C, Boitard C, Lahlou N, Atlan-Gepner C, Bouhanick B, Mogenet A, Nicolino M, Carel JC, Lecomte P, Maréchaud R, Bougnères P, Charbonnel B, Saï P. Oral insulin administration and residual beta-cell function in recent-onset type 1 diabetes: a multicentre randomised controlled trial. *Lancet* 2000; **356**: 545-549 [PMID: 10950231 DOI: 10.1016/s0140-6736(00)02579-4]

117   **Pozzilli P**, Gisella Cavallo M. Oral insulin and the induction of tolerance in man: reality or fantasy? *Diabetes Metab Res Rev* 2000; **16**: 306-307 [PMID: 11025554 DOI: 10.1002/1520-7560(200009/10)16:5<306::aid-dmrr150>3.0.co;2-j]

118   **Ergun-Longmire B**, Marker J, Zeidler A, Rapaport R, Raskin P, Bode B, Schatz D, Vargas A, Rogers D, Schwartz S, Malone J, Krischer J, Maclaren NK. Oral insulin therapy to prevent progression of immune-mediated (type 1) diabetes. *Ann N Y Acad Sci* 2004; **1029**: 260-277 [PMID: 15681764 DOI: 10.1196/annals.1309.057]

119   **Skyler JS**, Krischer JP, Wolfsdorf J, Cowie C, Palmer JP, Greenbaum C, Cuthbertson D, Rafkin-Mervis LE, Chase HP, Leschek E. Effects of oral insulin in relatives of patients with type 1 diabetes: The Diabetes Prevention Trial--Type 1. *Diabetes Care* 2005; **28**: 1068-1076 [PMID: 15855569 DOI: 10.2337/diacare.28.5.1068]

120   **Barker JM**, McFann KK, Orban T. Effect of oral insulin on insulin autoantibody levels in the Diabetes Prevention Trial Type 1 oral insulin study. *Diabetologia* 2007; **50**: 1603-1606 [PMID: 17583798 DOI: 10.1007/s00125-007-0694-0]

121   **Vehik K**, Cuthbertson D, Ruhlig H, Schatz DA, Peakman M, Krischer JP; DPT-1 and TrialNet Study Groups. Long-term outcome of individuals treated with oral insulin: diabetes prevention trial-type 1 (DPT-1) oral insulin trial. *Diabetes Care* 2011; **34**: 1585-1590 [PMID: 21610124 DOI: 10.2337/dc11-0523]

122   **Writing Committee for the Type 1 Diabetes TrialNet Oral Insulin Study Group**, Krischer JP, Schatz DA, Bundy B, Skyler JS, Greenbaum CJ. Effect of Oral Insulin on Prevention of Diabetes in Relatives of Patients With Type 1 Diabetes: A Randomized Clinical Trial. *JAMA* 2017; **318**: 1891-1902 [PMID: 29164254 DOI: 10.1001/jama.2017.17070]

123   **Bonifacio E**, Ziegler AG, Klingensmith G, Schober E, Bingley PJ, Rottenkolber M, Theil A, Eugster A, Puff R, Peplow C, Buettner F, Lange K, Hasford J, Achenbach P; Pre-POINT Study Group. Effects of high-dose oral insulin on immune responses in children at high risk for type 1 diabetes: the Pre-POINT randomized clinical trial. *JAMA* 2015; **313**: 1541-1549 [PMID: 25898052 DOI: 10.1001/jama.2015.2928]

124   **Ziegler AG**, Achenbach P, Berner R, Casteels K, Danne T, Gündert M, Hasford J, Hoffmann VS, Kordonouri O, Lange K, Elding Larsson H, Lundgren M, Snape MD, Szypowska A, Todd JA, Bonifacio E; GPPAD Study group. Oral insulin therapy for primary prevention of type 1 diabetes in infants with high genetic risk: the GPPAD-POInT (global platform for the prevention of autoimmune diabetes primary oral insulin trial) study protocol. *BMJ Open* 2019; **9**: e028578 [PMID: 31256036 DOI: 10.1136/bmjopen-2018-028578]

ORIGINAL ARTICLE

Case Control Study

Importance of serum phosphate in elderly patients with diabetes mellitus

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Abstract

BACKGROUND

Metabolic disturbances including changes in serum calcium, magnesium or phosphate (P) influence the prevalence of type 2 diabetes mellitus (DM). We assessed the importance of serum P in elderly patients with type 2 DM *vs* non-diabetes mellitus (non-DM) in relation to renal function.

AIM

To determine the association between serum P and serum glucose or insulin resistance in diabetic and non-diabetic patients.

METHODS

One hundred-ten subjects with a mean age of 69.02 ± 14.3 years were enrolled. Twenty-nine of the participants had type 2 DM (26.4%). The incidence of hypertension, smoking and receiving vitamin D (vitD) derivates were recorded. The participants were classified by both estimated glomerular filtration rate (eGFR) and albuminuria categories according to the Kidney Disease Improving Global Outcomes 2012 criteria.

RESULTS

We divided the patients in two groups according to the P cut-off point related to DM value. A comparison between high and low P showed that body mass index 30.2 ± 6.3 *vs* 28.1 ± 4.6 (*P* = 0.04), mean glucose 63.6 *vs* 50.2 (*P* = 0.03), uric acid 6.7 ± 1.6 *vs* 6.09 ± 1.7 (*P* = 0.05), mean intact-parathyroid hormone 68.06 *vs* 47.4 (*P* = 0.001), systolic blood pressure 147.4 ± 16.7 *vs* 140.2 ± 16.1 (*P* = 0.02), mean albuminuria 63.2 *vs* 50.6 (*P* = 0.04) and eGFR 45.6 ± 22.1 *vs* 55.4 ± 21.5 (*P* = 0.02) were significantly different. χ^2 tests showed a significant association between high P and DM, hypertension, receiving vitD, smoking and eGFR stage (χ^2 = 6.3, *P* =

0.01, $\chi^2 = 3.9$, $P = 0.03$, $\chi^2 = 6.9$, $P = 0.009$, $\chi^2 = 7.04$, $P = 0.01$ and $\chi^2 = 7.36$, $P = 0.04$, respectively). The adjusted model showed that older age, female gender and increased body mass index were significant predictors of type 2 DM when entering the covariates.

CONCLUSION
High serum P contributes to vascular and metabolic disturbances in elderly patients with type 2 DM and renal impairment.

Key Words: Serum phosphate; Diabetes mellitus; Renal disease; Old age; Albuminuria; Vitamin D

Core Tip: Despite high serum phosphate (P) being associated with hypertension, albuminuria, smoking, low estimated glomerular filtration rate and metabolic disorders, traditional factors including older age, female gender and high body mass index were proved to be potential predictors of type 2 diabetes mellitus. Serum P levels were similar in diabetic and non-diabetic patients and the association between serum P and serum glucose or insulin resistance was found to be non-significant in both diabetic and non-diabetic patients, which was discordant with previous reports, due to renal dysfunction.

INTRODUCTION

Patients with type 2 diabetes mellitus (DM) are a high risk group and metabolic disorders contribute to the prediction of morbidity and mortality in this population. Metabolic disturbances including changes in serum calcium, magnesium or phosphate (P) can explain why dyslipidemia, hyperglycemia and hyperuricemia, which are related to obesity, impact the progression to type 2 DM[1].

It has been reported that low serum levels of P are associated with increased insulin resistance in the healthy population[2]. Moreover, a previous experimental study using rats suggested that P depletion results in low insulin secretion by pancreatic beta cells, due to high intracellular calcium and inhibition of adenosine triphosphatase production[3]. Thus, it has been suggested that low serum P may disturb the regulation of serum glucose in non-DM with obesity[4].

Phosphate is essential for life, as it participates in the structure of cellular membranes as a material of nucleic acids, phospholipids and adenosine triphosphate. Additionally, P plays a crucial role in cellular signaling through reactions of phosphorylation. Homeostasis of P is affected by multiple interactions between the intestine, parathyroid glands, kidneys and bone.

Serum P levels are dependent on the absorption in the gut from dietary P, the excretion and reabsorption of P in the kidneys, and the movement of P between the extracellular and skeletal pools. Parathyroid hormone and fibroblast growth factor 23 play an important role in the regulation of serum P by mediating urinary P removal[5]. Elevated serum P is recognized as an independent predictor for advanced vascular disease in chronic kidney disease (CKD)[6]. However, epidemiological studies showed that all-cause mortality was independently related to increased serum P in all populations, even without CKD and serum P levels in the upper normal reference range[7,8].

In this study, we assessed the importance of serum P levels in elderly patients with type 2 DM compared to those without DM in relation to renal function clustering in CKD stages 1-4.

MATERIALS AND METHODS

Study subjects

This was a single-center cross-sectional study which included a total of one hundred-ten subjects. The participants were from the Department of Nephrology outpatient clinic of our Hospital, in which elderly non-dialysis patients are prevalent in accordance with most Nephrology Clinics worldwide. As the geriatric population continues to increase in most countries of the world (defined as age > 65 years) and the prevalence of renal disease rises with advancing age, nephrologists are usually confronted with an elderly patient population with co-morbidities and require ongoing care[9,10].

We studied sixty-seven males and forty-three females with a mean age of 69.02 ± 14.3 years after the exclusion of uncooperative patients and those who were younger than eighteen years of age. Subjects with established psychiatric symptomatology or dementia diagnosed by neuropsychologists were also excluded from the study, due mainly to invalid informed medical history or treatment.

Detailed individual medical histories and current pharmaceutical therapy were obtained from the participants. Twenty-nine of the participants had type 2 DM (26.4%) and eighty-one did not have DM (73.6%). The diabetics were taking the same hypoglycemic medications and both diabetics and non-diabetics were taking the same hypolipidemic medications. A total of seventy-five participants were hypertensive (68.2%) and thirty-five were non-hypertensive (31.8%). The hypertensive patients were receiving the same anti-hypertensive medications including beta-blockers, calcium channel blockers, and inhibitors of angiotensin II AT1 receptors. Forty subjects (36.4%) were taking vitamin D (vitD) and seventy (63.6%) were not taking vitD. None of the participants was taking P binders.

Demographic data including age, gender and lifestyle characteristics regarding physical activity, smoking and alcohol drinking were collected using a questionnaire. Fourteen of the participants were current smokers (12.7%), and ninety-six were non-smokers (87.3%). Non-drinkers were considered those who did not consume alcohol during the past month. The World Health Organization (WHO) recommendations for healthy adults were used to measure physical activity/inactivity[11].

Anthropometric measurements including body weight (to the nearest 0.1 kg) and height (to the nearest 0.1 cm) were recorded using an anthropometer (Seca, Hamburg, Germany). Body mass index (BMI) was calculated by dividing the body weight in kilograms by the square of the height in meters (kg/m^2) and categorized based on the WHO classification[12]. Waist circumference (WC), was measured from the midpoint between the top of iliac crest and the lower margin of the last palpable rib at the end of a normal expiration according to the WHO guidelines[13].

Biochemical measurements

Overnight fasting plasma glucose (normal range 65-110 mg/dL), creatinine (normal range 0.5-1.2 mg/dL), uric acid (normal range 2.6-6.0 mg/dL), calcium (normal range 8.1-10.4 mg/dL), P (normal range 2.5-4.5 mg/dL), triglycerides (normal range 40-150 mg/dL), low-density lipoprotein-cholesterol (normal range < 160 mg/dL) and high-density lipoprotein-cholesterol (normal range 35-80 mg/dL) were recorded from patient files using the latest results. A spectrophotometric technique using a Chemistry Analyzer (MINDRAY BS-200, Diamond Diagnostics, United States) was used for biochemical measurements.

The concentration of intact-parathyroid hormone (i-PTH) (normal range 18.5-88 pg/mL) and insulin (normal range 2.6-25 µU/mL) were measured by radioimmunoassays (CIS Bio International/France and BioSource Europe SA, Belgium, respectively). 25 hydroxyvitaminD_3 [25(OH) D_3] (normal range 30-100 ng/mL) was assessed using high-performance liquid chromatography[14].

The homeostasis model assessment of insulin resistance (HOMA-IR)[15] was used to calculate insulin resistance.

Urinary albumin and creatinine concentrations were measured by the Chemistry Analyzer using spot urine samples from the first morning void.

Definitions

Hypertension was defined as a mean systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 80 mmHg and/or participants who were taking antihypertensive therapy due to a pre-existing history of hypertension. We recommended the home two per day blood pressure measurements for SBP and DBP using an automatic sphygmomanometer (OMRON M4-I Co., Ltd., Kyoto, Japan).

Peripheral mean blood pressure (pMBP) was calculated as: pMBP = DBP + 0.4 (SBP - DBP). Pulse pressure was calculated as the difference between SBP and DBP.

The presence of CKD was defined according to the Kidney Disease Improving Global Outcomes (KDIGO) 2012 criteria for a duration more than 3 mo[16]. The estimated glomerular filtration rate (eGFR) was calculated using the CKD Epidemiology Collaboration equation and classified in 4 categories (1 to 4) according to KDIGO 2012 criteria, as we did not include patients in the fifth stage of CKD. We also classified our participants in stages based on albuminuria, which was defined as urinary albumin-to-creatinine ratio (ACR) ≥ 30 mg/g according to KDIGO 2012[16]. As the ACR correlates well with 24-h urinary albumin excretion, calculation of ACR using a spot urine sample was considered acceptable[17]. Primary renal diseases included hypertensive nephrosclerosis, diabetic nephropathy, interstitial nephritis and other/unknown.

Central or visceral obesity was determined by a WC ≥ 94 cm in men and ≥ 80 cm in women using the International Diabetes Federation criteria for the diagnosis of metabolic syndrome[18].

Statistical analysis
Data were presented as absolute numbers and frequencies for binary and categorical variables. Data were expressed as mean ± SD or as median value (interquartile range) for data that showed skewed distribution. The differences between mean values for two groups were assessed using the unpaired *t*-test and data that showed skewed distributions were compared using the Mann-Whitney *U*-test. Bivariate correlations between variables were defined by Spearman coefficient and comparisons between categorical variables were defined by χ^2 tests. A *P* value < 0.05 was considered statistically significant. We built a model using logistic regression analysis in order to investigate the predictive role of serum P on the manifestations of DM by adjusting for covariates. Additionally, we built models using linear regression analysis to determine the relationship between serum P and serum glucose levels adjusting for covariates. The SPSS 15.0 statistical package for Windows (SPSS Inc., Chicago, IL, United States) was used for statistical analysis.

RESULTS

Correlations
Bivariate correlations defined by the Spearman coefficient showed a significant positive correlation between serum P and serum glucose (*r* = 0.204, *P* = 0.03), uric acid (*r* = 0.195, *P* = 0.04), albuminuria (*r* = 0.192, *P* = 0.04), i-PTH (*r* = 0.239, *P* = 0.01) and pMBP (*r* = 0.214, *P* = 0.02), although the relationship between P and eGFR was found to be significantly inverse in all subjects (*r* = -0.224, *P* = 0.01). When including diabetics and non-diabetics separately we observed that the correlation between serum P and both serum glucose and HOMA-IR was non-significant in both groups.

Comparisons
We determined the differences between diabetics and non-diabetics and observed similar serum P levels between these groups.

The patients were then divided into two groups according to the receiver operating characteristic curve P cut-off point related to a DM value equal to 3.65 mg/dL (greater, *n* = 43 or lower, *n* = 67 than 3.65 mg/dL). Characteristics and differences between the two groups of patients are listed in Table 1. The comparison between high and low P showed: BMI: 30.2 ± 6.3 *vs* 28.1 ± 4.6 (*P* = 0.04), mean glucose: 63.6 *vs* 50.2 (*P* = 0.03), uric acid: 6.7 ± 1.6 *vs* 6.09 ± 1.7 (*P* = 0.05), mean i-PTH: 68.06 *vs* 47.4 (*P* = 0.001), SBP: 147.4 ± 16.7 *vs* 140.2 ± 16.1 (*P* = 0.02), pMBP: 108.02 ± 9.8 *vs* 103.6 ± 7.8 (*P* = 0.01), mean albuminuria: 63.2 *vs* 50.6 (*P* = 0.04), eGFR: 45.6 ± 22.1 *vs* 55.4 ± 21.5 (*P* = 0.02), WC: 108.09 ± 15.9 *vs* 102.8 ± 14.3 (*P* = 0.07) and mean HOMA-IR: 56.8 *vs* 54.6 (*P* = 0.7).

Categorical associations
χ^2 tests showed significant associations between high P and DM, hypertension and receiving vitD (χ^2 = 6.3, *P* = 0.01, χ^2 = 3.9, *P* = 0.03 and χ^2 = 6.9, *P* = 0.009, respectively). χ^2 tests between high P and both eGFR and smoking also revealed significant associations (χ^2 = 7.36, *P* = 0.04 and χ^2 = 7.04, *P* = 0.01, respectively). The relationship between higher serum P and albuminuria or high insulin resistance defined by HOMA-IR was found to be non-significant.

Table 1 The differences between groups of patients according to serum phosphate higher or lower than the cut-off point related to diabetes mellitus equal to 3.65 mg/dL

Characteristic	Patients with serum P > 3.65 mg/dL (*n* = 43), mean ± SD	Patients with serum P < 3.65 mg/dL (*n* = 67), mean ± SD	P value
Age (yr)	71.2 ± 12.8	67.6 ± 15.2	0.2
BMI (kg/m^2)	30.2 ± 6.3[a]	28.1 ± 4.6	0.04
WC (cm)	108.09 ± 15.9	102.8 ± 14.3	0.07
Uric acid (mg/dL)	6.7 ± 1.2[a]	6.09 ± 1.7	0.05
LDL-C (mg/dL)	102.2 ± 29.5	110.1 ± 32.1	0.2
HDL-C (mg/dL)	44.5 ± 13.2	47.1 ± 9.8	0.2
Triglycerides (mg/dL)	137.7 ± 54.4	129.01 ± 60.1	0.4
Calcium (mg/dL)	9.5 ± 0.6	9.5 ± 0.5	0.9
P (mg/dL)	4.2 ± 0.4[a]	3.2 ± 0.3	0.001
i-PTH (pg/mL)	mean rank = 68.06[a]	47.4	0.001
25(OH)D3 (ng/mL)	18.9 ± 12.7	20.8 ± 9.2	0.3
Glucose (mg/dL)	mean rank = 63.6[a]	50.28	0.03
Insulin (μU/mL)	11.6 ± 7.4	11.9 ± 8.7	0.8
HOMA-IR (mmol/L)	mean rank = 56.8	54.6	0.7
SBP (mmHg)	147.4 ± 16.7[a]	140.2 ± 16.1	0.02
DBP (mmHg)	mean rank = 62.08	51.28	0.07
pMBP (mmHg)	108.02 ± 9.8[a]	103.6 ± 7.8	0.01
PP (mmHg)	65.7 ± 16.2	61.01 ± 17.7	0.1
ACR (mg/g)	mean rank = 63.2[a]	50.6	0.04
eGFR (mL/min/1.73 m^2)	45.6 ± 22.1[a]	55.4 ± 21.5	0.02
Category variables	*n* (%)	*n* (%)	
DM (yes/no)	17 (39.5)/26 (60.5)[a]	12 (17.9)/55 (82.1)	0.01
Hypertension (yes/no)	34 (79.1)/9 (20.9)[a]	41 (61.2)/26 (38.8)	0.03
Receiving vitD (yes/no)	22 (51.2)/21 (48.8)[a]	18 (26.9)/49 (73.1)	0.009
Smoking (yes/no)	10 (23.3)/33 (76.7)[a]	4 (6)/63 (94)	0.01
Alcohol consumption (yes/no)	13 (30.2)/30 (69.8)	16 (23.9)/51 (76.1)	0.3
Physical activity (yes/no)	17 (39.5)/26 (60.5)	34 (50.7)/33 (49.3)	0.1
Classification based on eGFR:			0.04
eGFR > 90 mL/min/1.73 m^2	3 (7)[a]	6 (9)	
eGFR = 60-90 mL/min/1.73 m^2	7 (16.3)	18 (26.9)	
eGFR = 30-60 mL/min/1.73 m^2	21 (48.8)	37 (55.2)	
eGFR = 15-30 mL/min/1.73 m^2	12 (27.9)	6 (9)	

[a]$P < 0.05$. P: Phosphate; BMI: Body mass index; WC: Waist circumference; LDL-C: Low density lipoprotein cholesterol; HDL-C: High density lipoprotein cholesterol; i-PTH: Intact-parathyroid hormone; 25(OH)D$_3$: 25 hydroxyD$_3$; HOMA-IR: Homeostasis model assessment of insulin resistance; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; pMBP: Peripheral mean blood pressure; PP: Pulse pressure; ACR: Albumin-to-creatinine ratio; eGFR: Estimated glomerular filtration rate; DM: Diabetes mellitus.

Adjusted models

The logistic regression analysis model showed that older age, female gender and increased BMI were significant predictors for the manifestations of type 2 DM after entering hypertension, smoking, serum calcium and serum P levels as covariates (Table 2). Moreover, in non-diabetics we observed that only high BMI predicted

Table 2 Logistic regression analysis for predicting diabetes mellitus manifestations

Variables in model	*P* value	Odds ratio	Confidence interval
Age (yr)	0.003	1.07	1.02-1.13
Gender (male/female)	0.04	0.30	0.09–0.99
BMI (kg/m^2)	0.02	1.11	1.01–1.22
Hypertension (yes/no)	0.4	1.58	0.4–5.9
Smoking (yes/no)	0.08	3.7	0.8–17.02
Calcium (mg/dL)	0.2	1.6	0.7–3.7
P (mg/dL)	0.9	1.05	0.4–2.4

DM: Diabetes mellitus; BMI: Body mass index; P: Phosphate.

elevated serum glucose adjusting for age, gender, hypertension, smoking, eGFR, albuminuria, serum calcium and serum P (Table 3). In contrast, in diabetics we observed that only albuminuria can predict high serum glucose entering the same covariates (Table 4).

DISCUSSION

A previous study which included 162 patients with type 2 DM *vs* 82 hospitalized non-DM patients showed that serum P levels were lower in type 2 DM, due to the disturbance in metabolism[19]. In contrast, in the present study we did not observe reduced serum P in the diabetic group compared with the non-DM group. This discrepancy may be attributed to the fact that 69.1% of our subjects had low renal function defined by an eGFR less than 60 mL/min/1.73 m^2. The elimination of P depends on renal function, thus a positive P balance occurs in the early stage of renal dysfunction, although serum P levels mainly increase in advanced stages of CKD and remain elevated in patients in the end stage of renal disease without dialysis treatment. Indeed, as shown in Table 1, we noted a significant association between high serum P and eGFR and most of the patients with high serum P were classified in the third and fourth eGFR stage, although most of those with a low serum P were classified in the first, second and the third eGFR stage.

A previous study also reported a positive correlation between serum glucose and serum P in non-diabetics (*n* = 82), although in the type 2 diabetic group (*n* = 162) this correlation was found to be non-significant[19]. In contrast, we observed a positive correlation in all our subjects, but not separately in non-diabetics (*n* = 81) or diabetics (*n* = 29). Our findings are in agreement with those of the previous study regarding the diabetic patients, but not the non-diabetic patients, even though we included a similar number of non-diabetic participants to that in the previous study.

In addition, we noted a significant relationship between high serum P and the manifestations of DM divided according to the P cut-off point for DM. We also observed that the patients with higher serum P had more vascular and metabolic abnormalities than those with lower serum P, in agreement with previous reports[1,20]. The patients who had higher serum P had significantly higher blood pressure, BMI, uric acid, serum glucose, i-PTH, albuminuria and decreased eGFR in comparison to those with lower serum P. Furthermore, the relationship between high serum P and both hypertension and smoking was found to be significant. Indeed, it has already been noted that vascular calcification, arterial stiffness, cardiovascular mortality and progression of renal disease in patients with CKD or without CKD were correlated with higher serum P[21-23]. A previous study of the general population also showed that smokers have higher serum P levels[24].

It was observed that low serum P was combined with increased insulin resistance in the healthy population. A previous study of 881 non-diabetic subjects showed that low serum P levels were associated with high 2-h serum glucose and reduced insulin sensitivity[2]. However, in this study which included 81 non-diabetics we observed a non-significant correlation between serum P levels and insulin resistance defined by HOMA-IR. We also found mildly increased HOMA-IR combined with more central obesity in patients with higher serum P rather than in those with low serum P. We can

Table 3 Relation to serum glucose variables in our participants without diabetes mellitus (*n* = 81)

Variables in model	Beta	*t*	Sig.	Lower	Upper
Age (yr)	-0.02	-0.17	0.8	-0.28	0.24
Gender (male/female)	-0.03	-0.3	0.7	-7.8	5.6
BMI (kg/m^2)	0.4	3.9	0.001	0.5	1.6
Hypertension (yes/no)	0.02	0.2	0.8	-6.5	8.2
Smoking (yes/no)	0.04	0.3	0.7	-8.4	12.09
eGFR value (mL/min/1.73 m^2)	- 0.2	- 1.6	0.1	-0.3	0.03
ACR (mg/g)	0.03	0.2	0.7	-0.007	0.01
Calcium (mg/dL)	0.13	1.2	0.2	-2.4	9.7
P (mg/dL)	0.1	0.9	0.3	-2.7	7.7

Dependent variable: serum glucose. DM: Diabetes mellitus; BMI: Body mass index; eGFR: Estimated glomerular filtration rate; ACR: Albumin-to-creatinine ratio; P: Phosphate.

Table 4 Relation to serum glucose variables in our patients with diabetes mellitus (*n* = 29)

Variables in model	Beta	*t*	Sig.	Lower	Upper
Age (yr)	0.26	1.02	0.3	-1.06	3.1
Gender (male/female)	0.06	0.2	0.7	-30.5	39.8
BMI (kg/m^2)	0.3	1.2	0.2	-1.6	6.5
Hypertension (yes/no)	-0.2	-1.3	0.19	-70.04	15.5
Smoking (yes/no)	0.33	1.4	0.15	-12.7	74.4
eGFR value (mL/min/1.73 m^2)	- 0.06	-0.2	0.79	-1.1	0.86
ACR (mg/g)	0.4	2.2	0.03	0.003	0.09
Calcium (mg/dL)	0.2	1.1	0.2	-10.6	38.2
P (mg/dL)	-0.13	-0.6	0.5	-32.4	17.07

Dependent variable: serum glucose. DM: Diabetes mellitus; BMI: Body mass index; eGFR: Estimated glomerular filtration rate; ACR: Albumin-to-creatinine ratio; P: Phosphate.

explain this finding in our participants who had renal function impairment (69.1%) combined with a higher serum P. Decreased renal function itself is strongly associated with increased insulin resistance[25].

Moreover, in our study we observed a significant association between high serum P and receiving vitD, as it is known that vitD mediates the absorption and metabolism of P. However, it has been suggested that serum P changes during therapy with vitD derivates and the increase in P impedes and/or abolishes the beneficial effect of paricalcitol on endothelial function mostly in CKD patients[20].

Despite finding a significant non-adjusted association between high serum P and manifestations of DM, the adjusted model showed that older age, female gender and high BMI were significant predictors of DM manifestations, although high serum P was not. Furthermore, in the non-DM group, high BMI was revealed to be a unique significant predictor of high serum glucose levels, although in DM patients albuminuria was an important predictor of serum glucose including potential covariates as shown in Tables 3 and 4.

According to the findings of this study, despite the fact that high serum P was found to be associated with hypertension, albuminuria, smoking, low eGFR and metabolic disorders, the traditional factors including older age, female gender and high BMI were proved to be stronger predictors of type 2 DM manifestations rather than high serum P. Thus, those receiving vitD derivates require monitoring to prevent a rise in serum P resulting in the abolishment of the beneficial effect of vitD on

vascular endothelium.

Limitations
The main limitation of this study is the cross-sectional nature of the single-center design in combination with the small number of patients.

CONCLUSION
High serum P contributes to vascular and metabolic disturbances in elderly patients with type 2 DM and renal impairment. Serum P levels were similar in diabetics and non-diabetics and the relationship between serum P and serum glucose or insulin resistance was found to be non-significant in both diabetic and non-diabetic patients in contrast to previous reports, due to reduced renal function.

ARTICLE HIGHLIGHTS

Research background
Metabolic disorders contribute to the prediction of morbidity and mortality in patients with type 2 diabetes mellitus (type 2 DM). Changes in serum calcium, magnesium or P are related to the prevalence of type 2 DM mainly in combination with obesity.

Research motivation
We determined the importance of serum P levels in elderly patients with type 2 DM compared to those without DM in relation to renal function clustering in chronic kidney disease stages 1-4.

Research objectives
One hundred-ten subjects with a mean age of 69.02 ± 14.3 years were included. Twenty-nine participants had type 2 DM (26.4%).

Research methods
The participants were classified into both estimated glomerular filtration rate (eGFR) and albuminuria categories according to the Kidney Disease Improving Global Outcomes 2012 criteria. The incidence of hypertension, smoking and those receiving vitamin D derivates were recorded.

Research results
We divided the patients in two groups according to the P cut-off point related to type 2 DM. A significant association was observed between high P and type 2 DM, hypertension, receiving vitamin D, smoking and eGFR (χ^2 = 6.3, P = 0.01, χ^2 = 3.9, P = 0.03, χ^2 = 6.9, P = 0.009, χ^2 = 7.04, P = 0.01 and χ^2 = 7.36, P = 0.04, respectively). A multi-factorial model showed that older age, female gender and increased BMI were significant predictors of type 2 DM after entering the covariates.

Research conclusions
High serum P contributes to vascular and metabolic disturbances in elderly patients with type 2 DM and renal impairment.

Research perspectives
Compared with high serum P, traditional factors such as older age, female gender and high BMI were proved to be stronger predictors of type 2 DM.

REFERENCES

1   **Håglin L**, Törnkvist B, Bäckman L. Prediction of all-cause mortality in a patient population with hypertension and type 2 DM by using traditional risk factors and serum-phosphate,-calcium and-magnesium. *Acta Diabetol* 2007; **44**: 138-143 [PMID: 17721752 DOI: 10.1007/s00592-007-0254-6]

2   **Haap M**, Heller E, Thamer C, Tschritter O, Stefan N, Fritsche A. Association of serum phosphate levels with glucose tolerance, insulin sensitivity and insulin secretion in non-diabetic subjects. *Eur J Clin Nutr* 2006; **60**: 734-739 [PMID: 16391583 DOI: 10.1038/sj.ejcn.1602375]

3   **Zhou XJ**, Fadda GZ, Perna AF, Massry SG. Phosphate depletion impairs insulin secretion by pancreatic islets. *Kidney Int* 1991; **39**: 120-128 [PMID: 1848327 DOI: 10.1038/ki.1991.15]

4   **Håglin L**, Bäckman L, Törnkvist B. A structural equation model for assessment of links between changes in serum triglycerides, -urate, and -glucose and changes in serum calcium, -magnesium and -phosphate in type 2 diabetes and non-diabetes metabolism. *Cardiovasc Diabetol* 2011; **10**: 116 [PMID: 22192330 DOI: 10.1186/1475-2840-10-116]

5   **Sigrist M**, Tang M, Beaulieu M, Espino-Hernandez G, Er L, Djurdjev O, Levin A. Responsiveness of FGF-23 and mineral metabolism to altered dietary phosphate intake in chronic kidney disease (CKD): results of a randomized trial. *Nephrol Dial Transplant* 2013; **28**: 161-169 [PMID: 23024219 DOI: 10.1093/ndt/gfs405]

6   **Toussaint ND**, Pedagogos E, Tan SJ, Badve SV, Hawley CM, Perkovic V, Elder GJ. Phosphate in early chronic kidney disease: associations with clinical outcomes and a target to reduce cardiovascular risk. *Nephrology (Carlton)* 2012; **17**: 433-444 [PMID: 22574672 DOI: 10.1111/j.1440-1797.2012.01618.x]

7   **Eddington H**, Hoefield R, Sinha S, Chrysochou C, Lane B, Foley RN, Hegarty J, New J, O'Donoghue DJ, Middleton RJ, Kalra PA. Serum phosphate and mortality in patients with chronic kidney disease. *Clin J Am Soc Nephrol* 2010; **5**: 2251-2257 [PMID: 20688884 DOI: 10.2215/CJN.00810110]

8   **Tonelli M**, Sacks F, Pfeffer M, Gao Z, Curhan G; Cholesterol And Recurrent Events Trial Investigators. Relation between serum phosphate level and cardiovascular event rate in people with coronary disease. *Circulation* 2005; **112**: 2627-2633 [PMID: 16246962 DOI: 10.1161/CIRCULATIONAHA.105.553198]

9   **Sesso RC**, Lopes AA, Thomé FS, Lugon JR, Martins CT. Brazilian Chronic Dialysis Survey 2016. *J Bras Nefrol* 2017; **39**: 261-266 [PMID: 29044335 DOI: 10.5935/0101-2800.20170049]

10   **Rosner M**, Abdel-Rahman E, Williams ME; ASN Advisory Group on Geriatric Nephrology. Geriatric nephrology: responding to a growing challenge. *Clin J Am Soc Nephrol* 2010; **5**: 936-942 [PMID: 20185600 DOI: 10.2215/CJN.08731209]

11   **World Health Organization**. Global recommendations on Physical Activity for health. In: Diet, Physical Activity and Health. Available from: URL: https://www.who.int/dietphysicalactivity/factsheet_recommendations/en/

12   **World Health Organization**. Global Database on Body Mass Index: an Interactive Surveillance Tool for Monitoring Nutrition Transition. In: BMI classification. Geneva: World Health Organization; 2006.

13   **World Health Organization**. Waist Circumference and Waist-hip Ratio. Report of a WHO Expert Consultation, Geneva, 8-11 December 2008. In: Nutrition. Available from: URL: https://www.who.int/nutrition/publications/obesity/WHO_report_waistcircumference_and_waisthip_ratio/en/

14   **Neyestani TR**, Gharavi A, Kalayi A. Determination of serum 25-hydroxy cholecalciferol using high-performance liquid chromatography: a reliable tool for assessment of vitamin D status. *Int J Vitam Nutr Res* 2007; **77**: 341-346 [PMID: 18453319 DOI: 10.1024/0300-9831.77.5.341]

15   **Silva EA**, Flexa F, Zanella MT. Impact of abdominal fat and insulin resistance on arterial hypertension in non-obese women. *Arq Bras Endocrinol Metabol* 2009; **53**: 340-343 [PMID: 19578595 DOI: 10.1590/s0004-27302009000300007]

16   **Stevens PE**, Levin A; Kidney Disease: Improving Global Outcomes Chronic Kidney Disease Guideline Development Work Group Members. Evaluation and management of chronic kidney disease: synopsis of the kidney disease: improving global outcomes 2012 clinical practice guideline. *Ann Intern Med* 2013; **158**: 825-830 [PMID: 23732715 DOI: 10.7326/0003-4819-158-11-201306040-00007]

17   **Chen YT**, Hsu HJ, Hsu CK, Lee CC, Hsu KH, Sun CY, Chen CY, Chen YC, Yu YC, Wu IW. Correlation between spot and 24h proteinuria: Derivation and validation of equation to estimate daily proteinuria. *PLoS One* 2019; **14**: e0214614 [PMID: 30939176 DOI: 10.1371/journal.pone.0214614]

18   **Alberti G**, Zimmet P, Shaw J. Metabolic syndrome-a new world‑wide definition. A Consensus Statement from the International Diabetes Federation. 2006; **23**: 469-480 [DOI: 10.1111/j.1464-5491.2006.01858.x]

19   **Fang L**, Li X. [Level of serum phosphorus and adult type 2 diabetes mellitus]. *Zhong Nan Da Xue Xue Bao Yi Xue Ban* 2016; **41**: 502-506 [PMID: 27269925 DOI: 10.11817/j.issn.1672-7347.2016.05.009]

20   **Zoccali C**, Torino C, Curatola G, Panuccio V, Tripepi R, Pizzini P, Versace M, Bolignano D, Cutrupi S, Ghiadoni L, Thadhani R, Tripepi G, Mallamaci F. Serum phosphate modifies the vascular response to vitamin D receptor activation in chronic kidney disease (CKD) patients. *Nutr Metab Cardiovasc Dis* 2016; **26**: 581-589 [PMID: 27113290 DOI: 10.1016/j.numecd.2016.03.008]

21   **Adeney KL**, Siscovick DS, Ix JH, Seliger SL, Shlipak MG, Jenny NS, Kestenbaum BR. Association of serum phosphate with vascular and valvular calcification in moderate CKD. *J Am Soc Nephrol* 2009; **20**: 381-387 [PMID: 19073826 DOI: 10.1681/ASN.2008040349]

22   **Foley RN**, Collins AJ, Herzog CA, Ishani A, Kalra PA. Serum phosphorus levels associate with coronary atherosclerosis in young adults. *J Am Soc Nephrol* 2009; **20**: 397-404 [PMID: 18987306 DOI: 10.1681/ASN.2008020141]

23   **Dhingra R**, Sullivan LM, Fox CS, Wang TJ, D'Agostino RB Sr, Gaziano JM, Vasan RS. Relations of serum phosphorus and calcium levels to the incidence of cardiovascular disease in the community. *Arch Intern Med* 2007; **167**: 879-885 [PMID: 17502528 DOI: 10.1001/archinte.167.9.879]

24   **Håglin LM**, Törnkvist B, Bäckman LO. High serum phosphate and triglyceride levels in smoking women and men with CVD risk and type 2 diabetes. *Diabetol Metab Syndr* 2014; **6**: 39 [PMID: 24636522 DOI: 10.1186/1758-5996-6-39]

25   **Zammit AR**, Katz MJ, Derby C, Bitzer M, Lipton RB. Chronic Kidney Disease in Non-Diabetic Older Adults: Associated Roles of the Metabolic Syndrome, Inflammation, and Insulin Resistance. *PLoS One* 2015; **10**: e0139369 [PMID: 26431218 DOI: 10.1371/journal.pone.0139369]

ORIGINAL ARTICLE

Retrospective Cohort Study

Association between restrictive pulmonary disease and type 2 diabetes in Koreans: A cross-sectional study

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Abstract

BACKGROUND
Diabetes is a progressive disease that increases glucose levels in the blood. While studies have shown that patients with pulmonary disease (both obstructive and restrictive pulmonary disease) have a higher prevalence of type 2 diabetes mellitus (T2DM), there have been more studies on restrictive patterns than chronic obstructive pulmonary disease.

AIM
To assess whether restrictive and obstructive pulmonary diseases are associated with T2DM in Koreans.

METHODS
For our analysis, we used data from the Korea National Health and Nutrition Examination Survey. A total of 2830 subjects were included in this study. Spirometry results were categorized into three patterns: Normal, restrictive pulmonary disease (RPD), and obstructive pulmonary disease (OPD).

RESULTS
The factors used as diabetic indicators (*i.e.* homeostatic model assessment of insulin resistance, homeostatic model assessment of beta-cell function, glycated hemoglobin, and fasting insulin) were among the highest in RPD but not in OPD. Based on multivariate logistic regression analysis, subjects with RPD were found with an increased odds ratio [OR: 1.907, 95% confidence interval (CI): 1.110-3.277] for T2DM compared with subjects with normal pulmonary function, whereas in patients with OPD, the OR had not increased. Model 4, which adjusted for the variables that could affect diabetes and pulmonary disease, showed a significant increase in the T2DM OR to RPD (OR: 2.025, 95%CI: 1.264-3.244). On the other hand, no statistically significant difference was shown in OPD (OR: 0.982, 95%CI: 0.634-1.519).

CONCLUSION
RPD, not OPD, is highly associated with T2DM regardless of the risk factors of various T2DMs that can be confounds.

Key Words: Restrictive pulmonary disease; Obstructive pulmonary disease; Type 2 diabetes mellitus; Insulin resistance; Glycated hemoglobin; Koreans

Core Tip: This study was performed to assess whether restrictive and obstructive patterns of pulmonary disease and type 2 diabetes mellitus (T2DM) are associated with each other in Koreans. For our analysis, we used data from the Korea National Health and Nutrition Examination Survey. A total of 2830 subjects were included in this study. Spirometry results were categorized into three patterns: normal, restrictive, and obstructive pulmonary disease. Restrictive pulmonary disease, not obstructive disease, is highly relevant to T2DM regardless of other risk factors of various T2DMs that can be confounds.

INTRODUCTION

Diabetes is a progressive disease that increases glucose levels in the blood and has several pathogeneses, including insulin resistance in the liver and dysfunction of pancreas beta cells[1,2]. Type 2 diabetes mellitus(T2DM) is a complex disease associated with increased risk of multiple complications, such as peripheral circulation disease, and cardiovascular diseases such as stroke and coronary artery disease requiring intervention for treatment and prevention[3]. The cause of these cardiovascular diseases has been reported to be the increase in inflammation levels due to hyperglycemia and the weakening of cardiopulmonary functions[4]. Also, an increase of 1% of glycated hemoglobin (HbA1c), a blood sugar control factor, is known to increase the risk of cardiovascular disease by 28%[5]. Moreover, in a recent study, the risk of pulmonary dysfunction was higher in patients with impaired fasting glucose levels[6]. In addition, subjects with T2DM decreased forced expiratory volume in 1 s (FEV1) and forced vital capacity (FVC) regardless of race[7-9].

There are restrictive and obstructive pulmonary diseases in impaired pulmonary function[10]. Restrictive pulmonary disease (RPD) is reduced in both FVC and FEV1, resulting from a defect in thoracic compatibility[11]. On the other hand, obstructive pulmonary disease (OPD) is known to be caused by a significant reduction in FEV1, mainly due to airway blockages associated with smoking[12]. According to previous studies, impaired pulmonary function causes insulin resistance[13-15]. In addition, an increase in the inflammatory response derived from obesity causes insulin resistance and increases the risk of cardiovascular disease associated with obesity[16]. It was shown in previous studies that the prevalence rate of T2DM in chronic OPD patients is high[17,18], whereas it is more related to RPD than chronic OPD[19,20].

As such, the association between T2DM and impaired pulmonary function is not consistently explained. In addition, it is not clear whether this association is mediated by insulin resistance or by other factors. Therefore, based on the cross-sectional data from a large number of Korean subjects, this study examined the association between RPD and OPD with insulin resistance and T2DM.

MATERIALS AND METHODS

Data source and sampling

This study obtained data from the Korea National Health and Nutrition Examination Survey (KNHANES), 2015, a cross-sectional and nationally representative survey

conducted by the Korean Centers for Diseases Control and Prevention. The subjects were designated as those who responded to both the examination and the health survey among adults aged 40 or older who were subjected to the pulmonary function measurement. Among 7380 subjects that participated in KNHANES, 3008 subjects under 40 years of age, 1401 subjects who did not measure pulmonary function, 105 subjects who did not measure T2DM components, and 105 subjects who did not do the health survey were excluded. A total of 2830 participants were eligible for this study (Figure 1).

Measurements of variables

Covariates: Body mass index (BMI) was calculated by dividing weight (kg) by height (m). Waist circumference (WC) was measured at the midpoint between the bottom of the rib cage and the top of the lateral border of the iliac crest with full expiration. Blood samples were collected from subjects in the morning after overnight fasting and analyzed at a national central laboratory. Blood pressure was measured using a mercury sphygmomanometer in a seated position after a 10-min rest period. Two measurements were made for all subjects at 5-min intervals. An average of two measurements was used for the data analyses. Cigarette smoking condition was categorized as never smokers, ex-smokers, and current smokers, and drinking condition was dichotomized as current users and non-users. Physical examinations included HbA1c, C-reactive protein (hs-CRP), fasting insulin, fasting glucose, waist circumference, diastolic and systolic blood pressure, total cholesterol, low density lipoprotein, high density lipoprotein-cholesterol, and triglyceride measurement variables.

Measurement of pulmonary function

Pulmonary function was measured using a spirometer (model 2130; SensorMedics, Yorba Linda, CA, Untied States). Participants were classified according to respiratory patterns into a normal group (FEV 1/FVC ≥ 0.70, FVC ≥ 80% predicted), an OPD group (FEV 1/FVC < 0.70), and a RPD group (FVC < 80% predicted, FEV 1 / FVC ≥ 0.70)[21].

T2DM and insulin resistance

Homeostasis model assessment (HOMA) was used to calculate insulin resistance HOMA-IR and beta-cell function HOMA-beta indices using the formula: HOMA-IR = [fasting glucose (mg/dL) × fasting insulin (μU/mL)]/405 (> 2.5 indicating a high index of IR)[22] and HOMA-beta = [fasting insulin (lU/mL) 360]/[fasting glucose (mg/dL)-63][23].

Diabetes mellitus was defined by fasting glucose levels > 126 mg/dL in the health examination. Type 2 diabetes was distinguished from type 1 diabetes based on age at onset and treatment with insulin. Impaired fasting glucose (IFG) was defined as fasting glucose levels ≥ 100 mg/dL and < 125 mg/dL. Also, even if data such as fasting glucose and fasting insulin were normal, those who answered "yes" in the survey on whether they take diabetes drugs were classified as diabetic patients.

Statistical analysis

Since this study uses a complex sampling design, the weight given by the KNHANES has been applied. General characteristics were compared according to the pulmonary function and the prevalence of T2DM through the Chi-square test. A logistic regression analysis was used to analyze the association between pulmonary disease and T2DM, and *P* values < 0.05 were considered statistically significant. Data analysis uses the Statistic Package for Social Science 22.0 window version (Armonk, NY, United States).

RESULTS

In this study, the prevalence of RPD was 8.86% and OPD 14.20%. Significant differences by pulmonary disease were found in all variables except diastolic blood pressure and drinking status. Compared with those in the normal group, RPD and OPD subjects were of older age, with greater waist circumference, higher systolic blood pressure, and higher triglyceride. Also, smokers and men were higher in OPD than in normal and RPD. In terms of T2DM prevalence due to pulmonary disease, RPD accounted for 21.1%, the highest. In addition, the factors used as diabetic indicators, HOMA-IR, HOMA-beta, HbA1c, and fasting insulin, were all among the
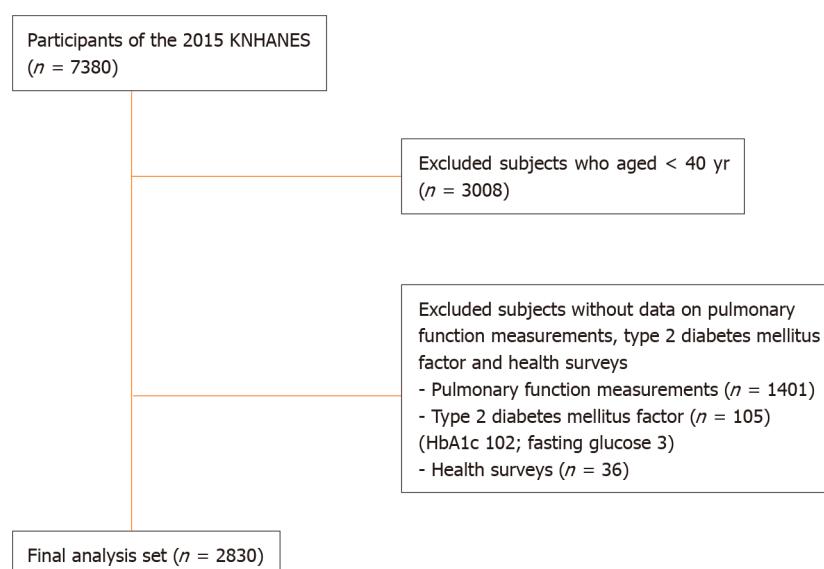
Figure 1 Subject selection from the Korea National Health and Nutrition Examination Survey 2015.

highest in the RPD, not in the OPD, compared to subjects with normal pulmonary function. hs-CRP, which indicates inflammation levels, was also the highest in the RPD (7.80 *vs* 9.84 *vs* 8.07) (Table 1).

Comparing pulmonary disease with fasting glucose levels, subjects with abnormal glucose levels (T2DM or IFG) had a higher prevalence rate of RPD and OPD compared to normal levels. In normal and IFG, the prevalence of RPD was significantly lower than that of OPD, but RPD was higher in T2DM (RPD/OPD: 6.2/11.5 *vs* 8.4/13.3 *vs* 18.1/15.9). In addition, HOMA-IR, HbA1c, and fasting insulin were higher with abnormal glucose levels, while HOMA-beta was significantly lower. The inflammatory factor Hs-CRP also higher in IFG and T2DM compared to normal.

To find out the association between pulmonary disease in subjects who do not have diabetes but are more likely to develop T2DM, multiple regression analyses were performed by dividing levels of normal, IFG, and T2DM groups (Table 2). Model 1, which adjusted for age and sex, showed that the probability of RPD was 1.453 times [95% confidence interval (CI): 1.059-1.995] for IFG and 3.621 times (95%CI: 2.316-5.663) for T2DM. However, Model 4, which adjusted for all variables that could be influential, showed 1.907 times (CI: 1.110-3.277) for T2DM. In contrast, the analysis of the association between OPD and IFG showed no significant association in any model (Table 3). Model 4, which adjusted for the variables that could affect diabetes and pulmonary disease, showed a significant increase in the T2DM odds ratio (OR) to the RPD (OR: 2.025, 95%CI: 1.264–3.244). On the other hand, no statistically significant difference was shown in OPD (OR: 0.982, 95%CI: 0.634–1.519) (Table 4).

DISCUSSION

This cross-sectional study is intended to identify the association of abnormal glucose in pulmonary disease. In particular, RPD was highly associated with increased ORs of T2DM regardless of major potential confounds, such as age and obesity factors. Thus, the main findings of this study are that T2DM is highly related to RPD but not OPD.

Pulmonary disease is associated with T2DM risk factors such as smoking, HbA1c, insulin resistance, hyperglycemia, and abdominal obesity, and these associations are particularly prominent in RPD[24,25]. The results of this study also showed significantly higher indicators of HbA1c, HOMA-IR, fasting glucose, and waist circumference in RPD compared to normal and OPD.

Although smoking is known to be a major cause of reduced pulmonary function[26], the results of this study show that it does not affect RPD. It has been confirmed to influence OPD. Other prior studies have shown that the association between RPD and T2DM prevalence rates is not significantly changed by smoking conditions, indicating that smoking has little influence.

HbA1c, measured for diagnosis of T2DM and monitoring glucose control, is a risk

Table 1 Characteristics of individuals with normal, restrictive, and obstructive pulmonary disease			
	Normal, *n* = 2177	RPD, *n* = 251	OPD, *n* = 402
Age (yr)[1]	53.50 ± 0.27[a]	57.79 ± 0.81[b]	62.40 ± 0.65[c]
Male (%)[1]	45.1[a]	50.8[a]	78.5[b]
T2DM (%)[1]	7.9[a]	21.1[b]	12.2[c]
HOMA-IR[1]	2.10 ± 0.07[a]	3.13 ± 0.41[b]	2.21 ± 0.14[a]
HOMA-beta[1]	78.51 ± 2.50[a]	83.13 ± 4.07[b]	76.23 ± 3.83[c]
HbA1c (%)[1]	5.71 ± 0.02[a]	6.14 ± 0.09[b]	5.93 ± 0.06[bc]
Hs-CRP (mg/L)[1]	1.07 ± 0.05[a]	1.79 ± 0.19[b]	1.48 ± 0.17[a]
Fasting insulin (UIU/mL)[1]	7.80 ± 0.16[a]	9.84 ± 0.61[b]	8.07 ± 0.43[a]
BMI (kg/m^2)[1]	24.08 ± 0.07[a]	25.67 ± 0.23[b]	24.08 ± 0.16[a]
Fasting glucose (mg/dL)[1]	101.99 ± 0.67[a]	116.33 ± 3.64[b]	105.85 ± 1.62[c]
Waist circumference (cm)[1]	82.97 ± 0.22[a]	87.59 ± 0.60[b]	86.29 ± 0.45[b]
SBP (mmHg)[1]	119.46 ± 0.42[a]	123.83 ± 1.27[b]	124.47 ± 0.85[b]
DBP (mmHg)[1]	77.22 ± 0.26	76.53 ± 0.86	76.27 ± 0.659
Total cholesterol (mg/dL)[1]	197.35 ± 0.89[a]	190.21 ± 2.59[b]	191.86 ± 2.43[b]
LDL-cholesterol (mg/dL)[1]	118.97 ± 0.81[a]	114.61 ± 2.21[a]	116.41 ± 2.37[b]
HDL-cholesterol (mg/dL)[1]	50.73 ± 0.34[a]	47.97 ± 0.88[b]	46.62 ± 0.68[b]
Triglyceride (mg/dL)[1]	148.73 ± 3.28[a]	155.62 ± 11.25[b]	157.15 ± 7.17[c]
Smoking status (%) (non-/ex-/current smoker)[1]	57.6/24.2/18.3[a]	56.7/25.3/18.0[a]	30.8/40.3/28.9[b]
Drinking alcohol status (%) (non-/current drinking)	25.6/74.4	30.5/69.5	23.3/76.7
FVC (% predicted)[1]	3.69 ± 0.02[a]	2.88 ± 0.04[b]	3.83 ± 0.07[a]
FEV1 (L)[1]	2.93 ± 0.02[a]	2.29 ± 0.04[b]	2.48 ± 0.05[c]
FEV1/FVC[1]	0.80 ± 0.00[a]	0.80 ± 0.00[a]	0.64 ± 0.00[b]
PEF (L/s)[1]	7.75 ± 0.06[a]	6.55 ± 0.12[b]	6.67 ± 0.11[b]

[1]$P < 0.05$ by ANOVA or chi-square test. [a,b,c]The same letters indicate non-significant difference between groups based on Bonferroni multiple comparison test. Data were presented as means ± SD or *n* (%). T2DM: Type 2 diabetes mellitus; Hs-CRP: Hs-C-reactive protein; BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; LDL-cholesterol: Low density lipoprotein-cholesterol; HDL-cholesterol: High density lipoprotein-cholesterol. FVC: Forced vital capacity; FEV1: Forced expiratory volume 1.

factor for cardiovascular disease[27]. In this study, the HbA1c level of RPD was the highest compared to normal and OPD (5.71 *vs* 6.14 *vs* 5.93). These results are consistent with the results of a prior study that showed a link between HbA1c and reduced pulmonary function in diabetics[28]. Moreover, the high level of HbA1c in healthy individuals means poor lung capacity, especially RPD[25].

Although pathological mechanisms for explaining the association between reduced pulmonary function and insulin resistance and T2DM have not been identified, there may be several common underlying causes. First, insulin resistance and hyperglycemia, the main risk factors for T2DM, caused decreased pulmonary function[29]. One study reported that insulin receptors exist in the lung pleura[30], and this insulin can change the physiology, which can promote deterioration of the respiratory muscle due to changes in glucose absorption in the thoracic muscle[31]. The results of this study indicate that HOMA-IR and fasting glucose figures are significantly higher in the RPD compared to normal and OPD, consistent with this hypothesis.

Second, the accumulation of fat in the abdominal cavity reduces lung volume and decreases the motion of the diaphragm, so pulmonary function is likely to be reduced[32,33]. The results of this study show that WC and BMI are significantly higher in RPD and OPD than in normal groups, supporting this hypothesis. However, there is

Table 2 Characteristics of individuals with pulmonary function in normal, impaired fasting glucose, and type 2 diabetic subjects

	Normal, *n* = 1608	IFG, *n* = 934	T2DM, *n* = 288
Age (yr)[1]	53.63 ± 0.31^a	56.23 ± 0.40^b	58.95 ± 0.71^c
Male (%)[1]	42.9 ± 1.3^a	58.2 ± 1.7^b	62.0 ± 3.5^b
RPD/OPD (%)[1]	$6.2/11.5^a$	$8.4/13.3^b$	$18.1/15.9^c$
HOMA-IR[1]	1.45 ± 0.03^a	2.49 ± 0.06^b	5.52 ± 0.58^c
HOMA-beta[1]	84.75 ± 1.58^a	76.38 ± 1.93^b	48.56 ± 3.96^c
HbA1c (%)[1]	5.47 ± 0.01^a	5.79 ± 0.02^b	7.61 ± 0.12^c
Hs-CRP (mg/L)[1]	0.94 ± 0.04^a	1.34 ± 0.09^b	2.04 ± 0.30^c
Fasting insulin (UIU/mL)[1]	6.42 ± 0.12^a	9.35 ± 0.24^b	13.02 ± 1.05^c
BMI (kg/m^2)[1]	23.63 ± 0.08^a	24.97 ± 0.11^b	25.19 ± 0.22^b
Fasting glucose (mg/dL)[1]	90.84 ± 0.16^a	107.44 ± 0.26^b	168.79 ± 3.34^c
Waist circumference (cm)[1]	81.52 ± 0.24^a	86.44 ± 0.31^b	88.32 ± 0.57^c
SBP (mmHg)[1]	117.92 ± 0.46^a	123.00 ± 0.58^b	126.55 ± 1.14^c
DBP (mmHg)[1]	76.39 ± 0.32^a	78.18 ± 0.39^b	77.10 ± 0.75^a
Total cholesterol (mg/dL)[1]	196.00 ± 0.96^a	198.67 ± 1.46^a	186.82 ± 2.73^b
LDL-cholesterol (mg/dL)[1]	118.77 ± 0.91^a	120.50 ± 1.30^a	107.90 ± 2.26^b
HDL-cholesterol (mg/dL)[1]	51.71 ± 0.39^a	48.25 ± 0.46^b	45.30 ± 0.72^c
Triglyceride (mg/dL)[1]	130.65 ± 3.08^a	166.09 ± 5.44^b	216.02 ± 15.20^c
Smoking status (%) (non-/ex-/current smoker)[1]	$59.9/22.1/18.0^a$	$46.6/32.0/21.4^b$	$45.6/31.7/22.8^b$
Drinking alcohol status (%) (non-/current drinking)	26.4/73.6	23.6/76.4	28.9/71.1
FVC (% predicted)[1]	3.61 ± 0.26^a	3.72 ± 0.04^b	3.56 ± 0.07^a
FEV1 (L)[1]	2.81 ± 0.02^a	2.86 ± 0.03^{ab}	2.72 ± 0.05^{ac}
FEV1/FVC[1]	0.78 ± 0.00^a	0.77 ± 0.00^b	0.77 ± 0.01^b
PEF (L/sec)[1]	7.42 ± 0.06^a	7.73 ± 0.09^b	7.48 ± 0.15^{ab}

[1]$P < 0.05$ by ANOVA or chi-square test. [a,b,c]The same letters indicate non-significant difference between groups based on Bonferroni multiple comparison test. Data were presented as means ± SD or *n* (%). IFG: Impaired fasting glucose; T2DM: Type 2 diabetes mellitus; Hs-CRP: Hs-C-reactive protein; BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; LDL-cholesterol: Low density lipoprotein-cholesterol; HDL-cholesterol: High density lipoprotein-cholesterol; FVC: Forced vital capacity; FEV1: Forced expiratory volume 1.

no statistically significant difference between the WC and BMI of RPD and OPD. In other words, abdominal obesity may be the basis for explaining the association between decreased pulmonary function and T2DM, but it does not seem to explain fully the relationship between RPD and T2DM.

Third, systemic inflammatory responses with insulin resistance lead to reduced pulmonary function and the development of diabetes[34]. Visceral fat, one of the risk factors for diabetes, affects the concentration of cytokines in the blood such as interleukin-6, adiponectin, leptin, and tumor necrosis factor-α, causing systemic inflammatory reactions and chronic low-grade inflammation reduced pulmonary function[35,36]. In this study, the hs-CRP, an indicator of systemic inflammatory reactions, was the highest in RPD, and the prior study is consistent with the findings that the increase in hs-CRP is highly related to pulmonary disease[37-39].

To summarize, there was a significant association between RPD and T2DM, whereas IFG was weak or not present. This suggests that T2DM is not a result of RPD, rather the cause of T2DM. Thus, it can be seen that risk factors, such as HOMA-IR, HbA1c, hyperglycemia, abdominal fat, and inflammatory index hs-CRP, are not sufficient in IFG to cause RPD compared to T2DM. Therefore, it would be worthwhile to examine the pulmonary function of IFG patients in future longitudinal studies according to the pattern of their T2DM progression.

Table 3 Odds ratios for pulmonary function according to the fasting glucose level by multivariate logistic regression analysis

	Pulmonary disease	Fasting glucose odds ratio (95%CI)	
		IFG (100-125)	T2DM (≥ 126)
Model 1	RPD	1.453 (1.059-1.995)[a]	3.621 (2.316-5.663)[b]
	OPD	1.199 (0.888-1.619)	1.744 (1.164-2.614)[a]
Model 2	RPD	1.282 (0.939-1.749)	2.890 (1.810-4.616)[b]
	OPD	0.725 (0.518-1.014)	0.821 (0.525-1.284)
Model 3	RPD	1.074 (0.781-1.476)	2.316 (1.438-3.729)[b]
	OPD	0.699 (0.498-0.982)	0.796 (0.501-1.267)
Model 4	RPD	0.934 (0.638-1.369)	1.907 (1.110-3.277)[a]
	OPD	0.722 (0.512-1.019)	0.782 (0.484-1.263)

[a]$P < 0.01$.

[b]$P < 0.001$. Model 1: Crude; Model 2: Adjusted for age, sex; Model 3: Adjusted for variables in Model 2 + body mass index, waist circumference, smoking status; Model 4: Adjusted for variables in Model 3 + C-reactive protein, homeostasis model assessment-IR. Reference category: Individuals with normal.

RPD: Restrictive pulmonary disease; OPD: Obstructive pulmonary disease; IFG: Impaired fasting glucose; T2DM: Type 2 diabetes mellitus.

Table 4 Odds ratios for type 2 diabetes mellitus according to the pulmonary function by multivariate logistic regression analysis

	Pulmonary disease	Odds ratio	95%CI
Model 1	RPD	3.127[b]	2.056-4.756
	OPD	1.631[a]	1.103-2.412
Model 2	RPD	2.580[b]	1.670-3.988
	OPD	1.033	0.673-1.584
Model 3	RPD	2.257[b]	1.465-3.475
	OPD	0.984	0.633-1.531
Model 4	RPD	2.025[a]	1.264-3.244
	OPD	0.982	0.634-1.519

[a]$P < 0.01$.

[b]$P < 0.001$. Model 1: Crude; Model 2: Adjusted for age, sex; Model 3: Adjusted for variables in model 2 + body mass index, waist circumference, smoking status; Model 4: Adjusted for variables in model 3 + C-reactive protein, homeostasis model assessment -IR. Reference category: Individuals with normal.

RPD: Restrictive pulmonary disease; OPD: Obstructive pulmonary disease.

Despite several meaningful findings of this study, there are several limitations. First, we could not use a specialized method to measure insulin resistance. However, it is reported that there is a high correlation between HOMA-IR and whole-body glucose absorption, measured using the euglycemic hyperinsulinemic clamp method. Second, because KNHANES's individuals who participated in this survey have relatively mild levels of comorbidities, a small number of severe-stage diabetics or pulmonary disease patients may affect the outcome analysis. In addition, the proportion of IFG or T2DM may have been somewhat high only for those aged 40 or older who conducted the pulmonary function tests. However, the strength of this data is that there is a high response rate, and it is thought that potential confounds will not have a significant impact on the results because it has been obtained from the representative information of a Korean population. Third, this study could not determine the temporal relationship because it was a cross-sectional design. This made it impossible to pinpoint the sequence of fundamental causes between pulmonary disease and T2DM. Therefore, it would be worthwhile to identify the mechanism between the two through future longitudinal studies.

CONCLUSION

This study was conducted to determine the association between pulmonary disease and T2DM. It was found that restrictive pulmonary function, not obstructive, is highly relevant to T2DM regardless of the various risk factors of T2DM that can be confounds.

ARTICLE HIGHLIGHTS

Research background

Previously, the association between type 2 diabetes mellitus (T2DM) and pulmonary disease was confirmed. Some studies found that T2DM is related to obstructive pulmonary disease (OPD), and others have shown that it is related to restrictive pulmonary disease (RPD).

Research motivation

T2DM and RPD are highly connected with T2DM, but research on causality between them is insufficient. Therefore, it is important to study this.

Research objectives

To find out the association between T2DM and pulmonary disease and to reveal its causal relationship.

Research methods

Korea National Health and Nutrition Examination Survey (KNHANES) is a survey research program conducted by the Korean Centers for Diseases Control and Prevention to assess the health and nutritional status of adults and children in Korea and to track changes over time. The survey combines interviews, physical examinations, and laboratory tests. KNHANES interview includes demographic, socioeconomic, dietary, and health-related questions. The examination component consists of medical, dental, and physiological measurements as well as laboratory tests administered by medical personnel, and all data are made anonymous and can be officially downloaded from the website. The KNHANES data are the official national disclosure data conducted annually. The data in this study are complex sampling design, using logistic regression analysis that is most appropriate to view the association between the variables recommended by the Korean Centers for Diseases Control.

Research results

Compared to OPD, the ratio of T2DM and its risk factors in restrictive RPD was very high. In addition, the analysis of pulmonary disease by fasting glucose level showed no significant difference in impaired fasting glucose group, and in T2DM, the probability of RPD occurring was 1.907 times higher than that of OPD. Also, the results of this study have significant association between RPD and T2DM, whereas impaired fasting glucose was weak or not present.

Research conclusions

RPD is highly relevant to T2DM regardless of risk factors. To summarize, this study suggests that RPD is not a cause of T2DM but rather a consequence of T2DM.

Research perspectives

In the future, a longitudinal study should identify changes in pulmonary function of impaired fasting glucose as it progresses.

REFERENCES

1   **Sonne DP**, Hemmingsen B. Comment on American Diabetes Association. *Standards of Medical Care in Diabetes-2017. Diabetes Care* 2017; **40**: S1-S135. Diabetes Care 2017; 40: e92-e93 [PMID: 28637892 DOI: 10.2337/dc17-0299]

2   **Hawkins D**, Bradberry JC, Cziraky MJ, Talbert RL, Bartels DW, Cerveny JD; National Pharmacy Cardiovascular Council. National Pharmacy Cardiovascular Council treatment guidelines for the management of type 2 diabetes mellitus: toward better patient outcomes and new roles for pharmacists.

Pharmacotherapy 2002; **22**: 436-444 [PMID: 11939679 DOI: 10.1592/phco.22.7.436.33667]

3   **Einarson TR**, Acs A, Ludwig C, Panton UH. Prevalence of cardiovascular disease in type 2 diabetes: a systematic literature review of scientific evidence from across the world in 2007-2017. *Cardiovasc Diabetol* 2018; **17**: 83 [PMID: 29884191 DOI: 10.1186/s12933-018-0728-6]

4   **Walther C**, Möbius-Winkler S, Linke A, Bruegel M, Thiery J, Schuler G, Halbrecht R. Regular exercise training compared with percutaneous intervention leads to a reduction of inflammatory markers and cardiovascular events in patients with coronary artery disease. *Eur J Cardiovasc Prev Rehabil* 2008; **15**: 107-112 [PMID: 18277195 DOI: 10.1097/HJR.0b013e3282f29aa6]

5   **Lind M**, Tuomilehto J, Uusitupa M, Nerman O, Eriksson J, Ilanne-Parikka P, Keinänen-Kiukaanniemi S, Peltonen M, Pivodic A, Lindström J. The association between HbA1c, fasting glucose, 1-hour glucose and 2-hour glucose during an oral glucose tolerance test and cardiovascular disease in individuals with elevated risk for diabetes. *PLoS One* 2014; **9**: e109506 [PMID: 25285769 DOI: 10.1371/journal.pone.0109506]

6   **Kim HK**, Kim CH, Jung YJ, Bae SJ, Choe J, Park JY, Lee KU. Association of restrictive ventilatory dysfunction with insulin resistance and type 2 diabetes in koreans. *Exp Clin Endocrinol Diabetes* 2011; **119**: 47-52 [PMID: 21246464 DOI: 10.1055/s-0030-1268467]

7   **Klein OL**, Aviles-Santa L, Cai J, Collard HR, Kanaya AM, Kaplan RC, Kinney GL, Mendes E, Smith L, Talavera G, Wu D, Daviglus M. Hispanics/Latinos With Type 2 Diabetes Have Functional and Symptomatic Pulmonary Impairment Mirroring Kidney Microangiopathy: Findings From the Hispanic Community Health Study/Study of Latinos (HCHS/SOL). *Diabetes Care* 2016; **39**: 2051-2057 [PMID: 27612502 DOI: 10.2337/dc16-1170]

8   **Lecube A**, Simó R, Pallayova M, Punjabi NM, López-Cano C, Turino C, Hernández C, Barbé F. Pulmonary Function and Sleep Breathing: Two New Targets for Type 2 Diabetes Care. *Endocr Rev* 2017; **38**: 550-573 [PMID: 28938479 DOI: 10.1210/er.2017-00173]

9   **Lee YJ**, Kim NK, Yang JY, Noh JH, Lee SS, Ko KS, Rhee BD, Kim DJ. Low pulmonary function in individuals with impaired fasting glucose: the 2007-2009 Korea national health and nutrition examination survey. *PLoS One* 2013; **8**: e76244 [PMID: 24086719 DOI: 10.1371/journal.pone.0076244]

10   **Schroeder EB**, Welch VL, Couper D, Nieto FJ, Liao D, Rosamond WD, Heiss G. Lung function and incident coronary heart disease: the Atherosclerosis Risk in Communities Study. *Am J Epidemiol* 2003; **158**: 1171-1181 [PMID: 14652302 DOI: 10.1093/aje/kwg276]

11   **Reeves-Hoche MK**, Meck R, Zwillich CW. Nasal CPAP: an objective evaluation of patient compliance. *Am J Respir Crit Care Med* 1994; **149**: 149-154 [PMID: 8111574 DOI: 10.1164/ajrccm.149.1.8111574]

12   **Tkacova R**. Systemic inflammation in chronic obstructive pulmonary disease: may adipose tissue play a role? Review of the literature and future perspectives. *Mediators Inflamm* 2010; **2010**: 585989 [PMID: 20414465 DOI: 10.1155/2010/585989]

13   **Engström G**, Janzon L. Risk of developing diabetes is inversely related to lung function: a population-based cohort study. *Diabet Med* 2002; **19**: 167-170 [PMID: 11874435 DOI: 10.1046/j.1464-5491.2002.00652.x]

14   **Engström G**, Hedblad B, Nilsson P, Wollmer P, Berglund G, Janzon L. Lung function, insulin resistance and incidence of cardiovascular disease: a longitudinal cohort study. *J Intern Med* 2003; **253**: 574-581 [PMID: 12702035 DOI: 10.1046/j.1365-2796.2003.01138.x]

15   **Litonjua AA**, Lazarus R, Sparrow D, Demolles D, Weiss ST. Lung function in type 2 diabetes: the Normative Aging Study. *Respir Med* 2005; **99**: 1583-1590 [PMID: 16291079 DOI: 10.1016/j.rmed.2005.03.023]

16   **Fantuzzi G**. Adipose tissue, adipokines, and inflammation. *J Allergy Clin Immunol* 2005; **115**: 911-9; quiz 920 [PMID: 15867843 DOI: 10.1016/j.jaci.2005.02.023]

17   **Vijayan VK**. Chronic obstructive pulmonary disease. *Indian J Med Res* 2013; **137**: 251-269 [PMID: 23563369]

18   **Stojkovikj J**, Zafirova-Ivanovska B, Kaeva B, Anastasova S, Angelovska I, Jovanovski S, Stojkovikj D. The Prevalence of Diabetes Mellitus in COPD Patients with Severe and Very Severe Stage of the Disease. *Open Access Maced J Med Sci* 2016; **4**: 253-258 [PMID: 27335596 DOI: 10.3889/oamjms.2016.060]

19   **Lange P**, Parner J, Schnohr P, Jensen G. Copenhagen City Heart Study: longitudinal analysis of ventilatory capacity in diabetic and nondiabetic adults. *Eur Respir J* 2002; **20**: 1406-1412 [PMID: 12503696 DOI: 10.1183/09031936.02.00050502]

20   **Kopf S**, Groener JB, Kender Z, Fleming T, Brune M, Riedinger C, Volk N, Herpel E, Pesta D, Szendrödi J, Wielpütz MO, Kauczor HU, Katus HA, Kreuter M, Nawroth PP. Breathlessness and Restrictive Lung Disease: An Important Diabetes-Related Feature in Patients with Type 2 Diabetes. *Respiration* 2018; **96**: 29-40 [PMID: 29874679 DOI: 10.1159/000488909]

21   **Knudson RJ**, Lebowitz MD, Holberg CJ, Burrows B. Changes in the normal maximal expiratory flow-volume curve with growth and aging. *Am Rev Respir Dis* 1983; **127**: 725-734 [PMID: 6859656 DOI: 10.1164/arrd.1983.127.6.725]

22   **Taura N**, Ichikawa T, Hamasaki K, Nakao K, Nishimura D, Goto T, Fukuta M, Kawashimo H, Fujimoto M, Kusumoto K, Motoyoshi Y, Shibata H, Abiru N, Yamasaki H, Eguchi K. Association between liver fibrosis and insulin sensitivity in chronic hepatitis C patients. *Am J Gastroenterol* 2006; **101**: 2752-2759 [PMID: 17026566 DOI: 10.1111/j.1572-0241.2006.00835.x]

23   **Dai CY**, Huang JF, Hsieh MY, Hou NJ, Lin ZY, Chen SC, Hsieh MY, Wang LY, Chang WY, Chuang WL, Yu ML. Insulin resistance predicts response to peginterferon-alpha/ribavirin combination therapy in chronic hepatitis C patients. *J Hepatol* 2009; **50**: 712-718 [PMID: 19231011 DOI: 10.1016/j.jhep.2008.12.017]

24   **Lim SY**, Rhee EJ, Sung KC. Metabolic syndrome, insulin resistance and systemic inflammation as risk factors for reduced lung function in Korean nonsmoking males. *J Korean Med Sci* 2010; **25**: 1480-1486 [PMID: 20890430 DOI: 10.3346/jkms.2010.25.10.1480]

25   **Oh IH**, Park JH, Lee CH, Park JS. The association of normal range glycated hemoglobin with restrictive lung pattern in the general population. *PLoS One* 2015; **10**: e0117725 [PMID: 25658743 DOI: 10.1371/journal.pone.0117725]

26   **Jayes L**, Haslam PL, Gratziou CG, Powell P, Britton J, Vardavas C, Jimenez-Ruiz C, Leonardi-Bee J;

Tobacco Control Committee of the European Respiratory Society. SmokeHaz: Systematic Reviews and Meta-analyses of the Effects of Smoking on Respiratory Health. *Chest* 2016; **150**: 164-179 [PMID: 27102185 DOI: 10.1016/j.chest.2016.03.060]

27   **Furuya A**, Suzuki S, Koga M, Oshima M, Amamiya S, Nakao A, Wada K, Okuhara K, Hayano S, Matsuo K, Tanahashi Y, Azuma H. HbA1c can be a useful glycemic control marker for patients with neonatal diabetes mellitus older than 20 wk of age. *Clin Chim Acta* 2014; **436**: 93-96 [PMID: 24854496 DOI: 10.1016/j.cca.2014.05.005]

28   **Ehrlich SF**, Quesenberry CP Jr, Van Den Eeden SK, Shan J, Ferrara A. Patients diagnosed with diabetes are at increased risk for asthma, chronic obstructive pulmonary disease, pulmonary fibrosis, and pneumonia but not lung cancer. *Diabetes Care* 2010; **33**: 55-60 [PMID: 19808918 DOI: 10.2337/dc09-0880]

29   **Lazarus R**, Sparrow D, Weiss ST. Baseline ventilatory function predicts the development of higher levels of fasting insulin and fasting insulin resistance index: the Normative Aging Study. *Eur Respir J* 1998; **12**: 641-645 [PMID: 9762793 DOI: 10.1183/09031936.98.12030641]

30   **Kouritas VK**, Hatzoglou C, Ioannou M, Gourgoulianis KI, Molyvdas PA. Insulin alters the permeability of sheep pleura. *Exp Clin Endocrinol Diabetes* 2010; **118**: 304-309 [PMID: 19856254 DOI: 10.1055/s-0029-1233452]

31   **Ribeiro MJ**, Sacramento JF, Gonzalez C, Guarino MP, Monteiro EC, Conde SV. Carotid body denervation prevents the development of insulin resistance and hypertension induced by hypercaloric diets. *Diabetes* 2013; **62**: 2905-2916 [PMID: 23530003 DOI: 10.2337/db12-1463]

32   **Canoy D**, Luben R, Welch A, Bingham S, Wareham N, Day N, Khaw KT. Abdominal obesity and respiratory function in men and women in the EPIC-Norfolk Study, United Kingdom. *Am J Epidemiol* 2004; **159**: 1140-1149 [PMID: 15191931 DOI: 10.1093/aje/kwh155]

33   **Salome CM**, King GG, Berend N. Physiology of obesity and effects on lung function. *J Appl Physiol (1985)* 2010; **108**: 206-211 [PMID: 19875713 DOI: 10.1152/japplphysiol.00694.2009]

34   **Mannino DM**, Ford ES, Redd SC. Obstructive and restrictive lung disease and markers of inflammation: data from the Third National Health and Nutrition Examination. *Am J Med* 2003; **114**: 758-762 [PMID: 12829203 DOI: 10.1016/s0002-9343(03)00185-2]

35   **Kern PA**, Ranganathan S, Li C, Wood L, Ranganathan G. Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. *Am J Physiol Endocrinol Metab* 2001; **280**: E745-E751 [PMID: 11287357 DOI: 10.1152/ajpendo.2001.280.5.E745]

36   **Lazarus R**, Sparrow D, Weiss ST. Impaired ventilatory function and elevated insulin levels in nondiabetic males: the Normative Aging Study. *Eur Respir J* 1998; **12**: 635-640 [PMID: 9762792 DOI: 10.1183/09031936.98.12030635]

37   **Rutter MK**, Meigs JB, Sullivan LM, D'Agostino RB Sr, Wilson PW. C-reactive protein, the metabolic syndrome, and prediction of cardiovascular events in the Framingham Offspring Study. *Circulation* 2004; **110**: 380-385 [PMID: 15262834 DOI: 10.1161/01.CIR.0000136581.59584.0E]

38   **Jung DH**, Shim JY, Ahn HY, Lee HR, Lee JH, Lee YJ. Relationship of body composition and C-reactive protein with pulmonary function. *Respir Med* 2010; **104**: 1197-1203 [PMID: 20299196 DOI: 10.1016/j.rmed.2010.02.014]

39   **Okita K**, Iwahashi H, Kozawa J, Okauchi Y, Funahashi T, Imagawa A, Shimomura I. Homeostasis model assessment of insulin resistance for evaluating insulin sensitivity in patients with type 2 diabetes on insulin therapy. *Endocr J* 2013; **60**: 283-290 [PMID: 23149658 DOI: 10.1507/endocrj.ej12-0320]

Retrospective Cohort Study

Comparison of clinical features and outcomes in peritoneal dialysis-associated peritonitis patients with and without diabetes: A multicenter retrospective cohort study

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Abstract

BACKGROUND
The number of end-stage renal disease patients with diabetes mellitus (DM) who are undergoing peritoneal dialysis is increasing. Peritoneal dialysis-associated peritonitis (PDAP) is a serious complication of peritoneal dialysis leading to technical failure and increased mortality in patients undergoing peritoneal dialysis. The profile of clinical symptoms, distribution of pathogenic organisms, and response of PDAP to medical management in the subset of end-stage renal disease patients with DM have not been reported previously. Discrepant results have been found in long-term prognostic outcomes of PDAP in patients with DM. We inferred that DM is associated with bad outcomes in PDAP patients.

AIM
To compare the clinical features and outcomes of PDAP between patients with DM and those without.

METHODS
In this multicenter retrospective cohort study, we enrolled patients who had at

least one episode of PDAP during the study period. The patients were followed for a median of 31.1 mo. They were divided into a DM group and a non-DM group. Clinical features, therapeutic outcomes, and long-term prognostic outcomes were compared between the two groups. Risk factors associated with therapeutic outcomes of PDAP were analyzed using multivariable logistic regression. A Cox proportional hazards model was constructed to examine the influence of DM on patient survival and incidence of technical failure.

RESULTS

Overall, 373 episodes occurred in the DM group (n = 214) and 692 episodes occurred in the non-DM group (n = 395). The rates of abdominal pain and fever were similar in the two groups (P > 0.05). The DM group had more infections with coagulase-negative Staphylococcus and less infections with *Escherichia coli* (*E. coli*) as compared to the non-DM group (P < 0.05). Multivariate logistic regression analysis revealed no association between the presence of diabetes and rates of complete cure, catheter removal, PDAP-related death, or relapse of PDAP (P > 0.05). Patients in the DM group were older and had a higher burden of cardiovascular disease, with lower level of serum albumin, but a higher estimated glomerular filtration rate (P < 0.05). Cox proportional hazards model confirmed that the presence of diabetes was a significant predictor of all-cause mortality (hazard ratio = 1.531, 95% confidence interval: 1.091-2.148, P < 0.05), but did not predict the occurrence of technical failure (P > 0.05).

CONCLUSION

PDAP patients with diabetes have similar symptomology and are predisposed to coagulase-negative Staphylococcus but not *E. coli* infection compared those without. Diabetes is associated with higher all-cause mortality but not therapeutic outcomes of PDAP.

Key Words: Diabetes mellitus; Mortality; Peritoneal dialysis; Peritoneal dialysis-associated peritonitis; Technical failure

Core Tip: We for the first time confirmed that the symptoms of peritoneal dialysis-associated peritonitis in the diabetes mellitus group were the same as those in the non-diabetes mellitus group. This is the first multicenter retrospective cohort study to examine the relationship between diabetes mellitus and long-term outcome in peritoneal dialysis-associated peritonitis patients. It is also the first study to analyze the profile of distribution of pathogenic organisms and response of peritoneal dialysis-associated peritonitis to medical management in the subset of end-stage renal disease patients with diabetes mellitus. We found that diabetes mellitus was inclined to infection with coagulase-negative Staphylococcus but not *Escherichia coli*. Diabetes mellitus was associated with higher all-cause mortality but not with adverse therapeutic outcome of peritoneal dialysis-associated peritonitis.

INTRODUCTION

The prevalence of diabetes mellitus (DM) in the general population is increasing so rapidly that diabetic nephropathy is now the leading cause of end-stage renal disease (ESRD) worldwide[1,2]. ESRD patients with DM who are undergoing renal replacement therapy in the form of dialysis pose certain group specific challenges to the overall

well-being of these patients[3,4]. Adequate vascular access for hemodialysis is often a concern in ESRD patients with diabetes, consequently many patients may have to opt for peritoneal dialysis.

Peritoneal dialysis-associated peritonitis (PDAP) is a common and serious complication that not only leads to technical failure[5,6], but is also associated with increased mortality in patients undergoing peritoneal dialysis (PD)[7]. Moreover, PDAP-related death constitutes a major chunk of all-cause mortality in patients undergoing PD[8]. Some studies suggest that DM is a risk factor for PDAP[9,10], therefore, PDAP in patients with both ESRD and DM should draw careful attention. We hypothesized that there may be some differences in clinical features (symptoms and pathogens) and prognosis of PDAP between DM and non-DM patients. However, only few studies have analyzed the long-term prognostic outcomes of PDAP in patients with DM, often with discrepant results. Some studies concluded that DM was not a risk factor for death or technical failure[11,12] while others found DM to be a risk factor for all-cause death[13], yet again some researchers like Tsai *et al*[14] found DM to be a significant risk factor for a combined outcome of death and catheter removal. More importantly, as far as we know, the profile of clinical symptoms, distribution of pathogenic organisms, and response of PDAP to medical management in the subset of ESRD patients with DM has not been reported previously.

To clarify the above issues, a large multicenter retrospective cohort study was performed to compare the clinical features (symptoms and pathogens) and outcomes (therapeutic outcomes and long-term prognostic outcomes) of PDAP in ESRD patients with DM with those without.

MATERIALS AND METHODS

Participants
This multicenter study was performed in Northeast China; the participating centers were The Second Hospital of Jilin University, The First Hospital of Jilin University-Eastern Division, Jilin FAW General Hospital, and Jilin Central Hospital. All PD patients who developed PDAP during the study period from January 1, 2013 to June 30, 2019 were recruited and followed until December 31, 2019. Patients with incomplete records, patients younger than 18 years, and those with chronic liver disease at initiation of PD were excluded from the study. Patients on immu-nosuppressant medications or steroids or with a history of intake of the same within the last 3 mo were also excluded from the study. We adhered to all the ethical requirements for retrospective observational studies at our center. Individual informed consent was waived given that the study was retrospective and non-interventional by design. We used a de-identified dataset. Diabetes was diagnosed according to American Diabetes Association criteria 2014[15]. According to the status of diagnosis of DM at initiation of PD, the patients were divided into a DM group and a non-DM group. They were followed until any of the following events: Death, a change to HD, renal transplantation, dropout, transfer to other centers, diagnosis with DM after initiation of PD, or until 31 December 2019.

Main clinical management
Double-cuff Tenckhoff straight catheters and integrated Y-sets were used for PD treatment. PD trainings were conducted by experienced and educated physicians and nurses. The patients were asked to report back if they experienced cloudy effluent or abdominal pain. PD effluent was sent for bacterial and fungal culture by inoculation into blood culture media, and observed for at least 72 h to document pathogens. The diagnosis of PDAP required any two of the following features: (1) Clinical features consistent with peritonitis, *i.e.*, abdominal pain and/or cloudy dialysis effluent; (2) dialysis effluent white cell count > 100/μL or > 0.1×10^9/L (after a dwell time of at least 2 h), with > 50% polymorphonuclear cells; and (3) positive dialysis effluent culture[16]. All patients suspected of having PDAP were managed as per the International Society for Peritoneal Dialysis recommendation, which includes treatment with empiric intra-peritoneal antibiotics at presentation covering both Gram-positive and Gram-negative organisms, after taking culture samples[16]. Subsequent choice of antibiotics was directed by the effluent culture and sensitivity results.

Data collection

Baseline data collected at the time of first episode of PDAP encompassed demographic data [age, gender, presence of DM, and history of cardiovascular disease (CVD)], timing of PDAP episodes, clinical and biochemical data, fever, abdominal pain, PD cell count on admission, 24 h urine output, serum white cell count, hemoglobin, serum albumin, blood urea nitrogen, serum creatinine, and estimated glomerular filtration rate (eGFR). Biochemical measurements were performed by standard laboratory techniques. The culture results of effluent samples were subcategorized into mono-microbial (Gram-positive, Gram-negative, fungal, and mycobacterial organisms), polymicrobial, culture-negative, and no culture. Patients with ≥ 2 cultured pathogens were considered to have polymicrobial peritonitis.

Definitions of study outcomes

Therapeutic outcomes of medical management of PDAP included complete cure, catheter removal, PDAP-related death, and relapse. Complete cure was defined as complete resolution of PDAP by antibiotics alone without relapse or recurrence within 4 wk of completion of therapy[17]. PDAP-related death was defined as patient's death with peritonitis occurring within 30 d[8]. Relapse was defined as an episode occurring within 4 wk of completion of therapy with the same organism being isolated in effluent culture as in the previous episode[16].

Long-term prognostic outcomes of PDAP included continued PD, technical failure, and all-cause mortality. All-cause mortality was the primary endpoint in the patient survival analysis. If a patient died within 4 wk after switching over to hemodialysis, the death was attributed to PD because these early deaths are considered to reflect the health status of the patient during PD therapy[18]. Technical failure was defined as a switch to HD for at least 3 mo due to any reason[19].

Statistical analysis

Normally distributed parametric continuous variables are represented as the mean ± standard deviation, and were compared by Student's *t*-test. Continuous variables with a non-normal distribution are represented as medians (Q1-Q3), and were compared using Wilcoxon's rank-sum test. Categorical variables are represented as frequencies (percentages) and were compared using the chi-square (χ^2) test. The risk factors associated with therapeutic outcomes of PDAP were analyzed using multivariable logistic regression. Kaplan-Meier survival curves were constructed to evaluate cumulative hazard of all-cause mortality and technical failure between the two groups, and differences in the survival distribution was assessed by log rank test. Cox proportional hazard analysis was used to analyze the relationship between DM and all-cause mortality. Data were analyzed using SPSS (version 22.0, IBM, New York, United States). A P value < 0.05 was considered statistically significant. All the artworks were created using GraphPad Prism (version 8.0).

The statistical methods of this study were reviewed by Su-Yan Tian from the First Hospital of Jilin University.

RESULTS

Study population and baseline characteristics of 1065 PDAP episodes

A total of 1145 episodes of PDAP occurred in 660 patients from four PD centers in Northeast China during the study period. Finally, 1065 episodes of peritonitis in 609 patients were included in this study (Figure 1). Patients in the DM group had significantly lower levels of serum albumin and serum phosphorus, but a higher level of eGFR (P < 0.05). There was no difference in the frequency or distribution of symptoms such as fever and abdominal pain between DM group and non-DM group (P > 0.05) (Table 1).

Causative organisms of 1065 PDAP episodes

Among the 1065 PDAP episodes, 373 (35%) episodes occurred in the DM group. The differential distribution of causative organisms of PDAP between the two groups is shown in Table 2. The incidence of infection by Gram-positive bacteria, especially coagulase-negative Staphylococcus (CNS), was significantly higher in patients in the DM group (P < 0.05) while the incidence of *E. coli* and *Pseudomonas aeruginosa* infection was significantly higher in the non-DM group (P < 0.05). No significant difference was found in other organisms between the two groups (P > 0.05).

Table 1 Clinical manifestations and laboratory parameters of 1065 peritoneal dialysis-associated peritonitis events

Index	DM (*n* = 373)	Non-DM (*n* = 692)	*P*
Clinical manifestation			
Fever	115 (30.8)	253 (36.6)	0.061
Abdominal pain	300 (80.4)	552 (79.8)	0.797
PD cell count on admission(/μL)	1920 (620, 5350)	1847 (613, 4974)	0.791
Laboratory test			
WBC (10^{12}/L)	8.27 (6.57, 10.98)	8.29 (6.09, 11.43)	0.903
Hb (g/L)	97 (84, 109)	99 (85, 113)	0.128
Alb (g/dL)	28.24 ± 6.24	29.42 ± 6.29	0.003
BUN (mmol/L)	14.85 (10.79, 20.16)	15.68 (12.01, 19.92)	0.090
Scr (μmol/L)	672.66 (511.00, 854.50)	735.60 (511.00, 954.35)	0
eGFR	6.29 (4.68, 8.38)	5.70 (4.35, 7.28)	0

DM: Diabetes mellitus; PD: Peritoneal dialysis; WBC: White cell count; Hb: Hemoglobin; Alb: Albumin; BUN: Blood urea nitrogen; Scr: Serum creatinine; eGFR: Estimated glomerular filtration rate.

Table 2 Causative organisms of 1065 peritoneal dialysis-associated peritonitis

Organism (*n*, %)	DM (*n* = 373)	Non-DM (*n* = 692)	*P*
Gram-positive	172 (46.1)	259 (37.4)	0.006
Coagulase-negative staphylococcus	103 (27.6)	130 (18.8)	0.001
Staphylococcus aureus	22 (5.9)	26 (3.8)	0.108
Streptococcus species	30 (8.0)	59 (8.5)	0.786
Enterococcus species	8 (2.1)	11 (1.6)	0.514
Other gram-positive	9 (2.4)	33 (4.8)	0.060
Gram-negative	74 (19.8)	170 (24.6)	0.080
Escherichia coli	26 (6.7)	73 (10.5)	0.038
Klebsiella species	6 (1.6)	20 (2.9)	0.196
Acinetobacter baumannii	7 (1.9)	17 (2.5)	0.543
Pseudomonas aeruginosa	5 (1.3)	27 (3.9)	0.020
Other gram-negative	31 (8.3)	32 (4.6)	0.015
Fungi	13 (3.5)	30 (4.3)	0.501
Mycobacterium tuberculosis	2 (0.5)	8 (1.2)	0.508
Polymicrobial	24 (6.4)	55 (7.9)	0.369
Culture-negative	84 (22.5)	163 (23.6)	0.703
No culture	4 (1.1)	7 (1.0)	1.000

DM: Diabetes mellitus.

Therapeutic outcomes of 1065 PDAP episodes

There was also no significant difference in the outcomes including rates of complete cure, catheter removal, PDAP-related death, and relapse of PDAP between the two groups (*P* > 0.05) (Figure 2A). By using a multivariable logistic regression model, we did not find diabetes to be a significant risk factor for adverse therapeutic outcomes of PDAP (*P* > 0.05) (Figure 2B).
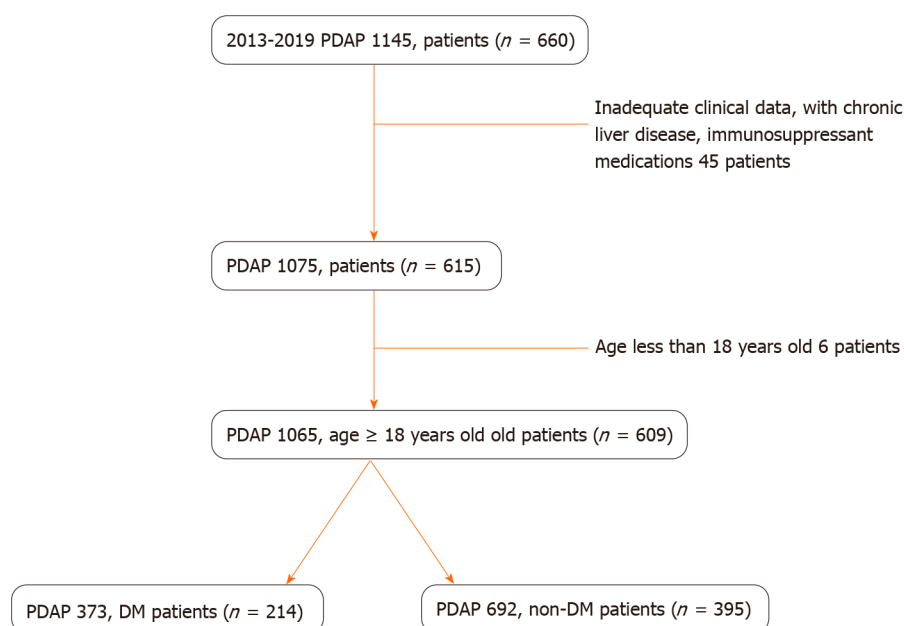
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│ 2013-2019 PDAP 1145, patients (n = 660) │
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                    │          Inadequate clinical data, with chronic
                    │          liver disease, immunosuppressant
                    │          medications 45 patients
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│      PDAP 1075, patients (n = 615)      │
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                    │          Age less than 18 years old 6 patients
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│  PDAP 1065, age ≥ 18 years old old patients (n = 609)  │
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│ PDAP 373, DM patients (n = 214)│  │ PDAP 692, non-DM patients (n = 395)│
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Figure 1 Flowchart of cohort establishment. PDAP: Peritoneal dialysis-associated peritonitis; DM: Diabetes mellitus.

Baseline characteristics of 609 patients with PDAP

The patients were followed for a median of 31.1 mo (interquartile range, 16.5-49.6 mo). Baseline demographic characteristics and laboratory test parameters of patients with and without DM were compared and are presented in Table 3. Compared with patients without DM, the patients with DM were older and had a higher burden of CVD ($P < 0.05$). Patients in the DM groups had a lower level of serum albumin, but a higher level of eGFR ($P < 0.05$).

Long-term prognostic outcomes of 609 patients with PDAP

One hundred and fifty (24.6%) patients died during the study period, of whom 71 belonged to the DM group and 79 to the non-DM group. The reasons for death included PDAP, cardiovascular death, cerebrovascular death, and others. Compared to the non-DM group, the all-cause mortality rate in the DM group was significantly higher, and correspondingly the rate of continuing on dialysis was significantly lower ($P < 0.05$). One hundred and thirty-four (22.0%) patients experienced technical failure, of whom 42 were in the DM group and 92 in the non-DM group. There was no significant difference in the rates of technical failure between the two groups ($P > 0.05$) (Figure 3A).

Kaplan-Meier survival curves showed that the DM group had a higher all-cause mortality rate compared to the non-DM group ($P < 0.05$) (Figure 3B). There was no significant association between the occurrence of diabetes and incident rates of technical failure ($P > 0.05$) (Figure 3C).

Cox regression analysis was used to analyze the relationship between DM and all-cause mortality. It was found that DM was a significant independent risk factor for all-cause mortality (hazard ratio = 1.531, 95% confidence interval: 1.091-2.148, $P < 0.05$) (Figure 3D).

DISCUSSION

The present study aimed to explore differences in the clinical features and outcomes in PDAP patients with and without diabetes as we hypothesized. We found that the symptoms of PDAP between the DM group and non-DM group were similar; the DM group had more infections with CNS and less infections with *E. coli* as compared to the non-DM group; the therapeutic outcomes of PDAP including complete cure, catheter removal, PDAP-related death, and relapse were comparable between the two groups; DM was an independent risk factor of all-cause mortality but not technique failure in PDAP patients.

Table 3 Demographic characteristics and clinical data of 609 peritoneal dialysis-associated peritonitis patients

Variable	DM (*n* = 214)	Non-DM (*n* = 395)	*P*
Demographic characteristics			
Age (yr)	61 (54, 68)	55 (42, 68)	0
Gender (male, *n*, %)	117 (54.7)	185 (48.6)	0.065
No. of PDAP episodes, *n* (%)			0.596
1	127 (59.3)	230 (58.2)	
2	51 (23.8)	86 (21.8)	
≥ 3	36 (16.8)	79 (20.0)	
CVD	77 (36.0)	104 (26.3)	0.013
Laboratory test			
WBC (10^{12}/L)	8.41 (6.90,10.62)	8.26 (5.99,11.29)	0.411
Hb (g/L)	96 (80, 108)	98 (83, 111)	0.089
Alb (g/dL)	28.34 ± 6.74	29.60 ± 5.88	0.017
BUN (mmol/L)	14.9 (10.2, 20.5)	16.0 (12.5, 20.2)	0.062
Scr (µmol/L)	654.7 (480.0, 858.0)	745.3 (575.2, 972.2)	0.001
eGFR	6.46 (4.61, 8.55)	5.51 (4.23, 7.17)	0
PD cell count on admission(/µL)	1799.5 (571.0, 5040.0)	2040 (739.0, 5219.5)	0.330

DM: Diabetes mellitus; PDAP: Peritoneal dialysis-associated peritonitis; CVD: Cardiovascular disease; WBC: White cell count; Hb: Hemoglobin; Alb: Albumin; BUN: Blood urea nitrogen; Scr: Serum creatinine; eGFR: Estimated glomerular filtration rate; PD: Peritoneal dialysis.

We found no difference in the symptomatology of PDAP between the two groups. Theoretically, the symptoms of PDAP in diabetic patients may be atypical due to the presence of concomitant peripheral and autonomic neuropathy[14]. However, we confirmed that the symptoms of PDAP in the DM group were the same as those in the non-DM group for the first time.

To the best of our knowledge, no prior study has compared differences in microbial isolates on culture of effluent fluid between PDAP patients with and without diabetes. Our study characterized the microbiological etiology of PDAP in patients with DM over a period of 7 years. We found important differences in the distribution of organisms responsible for PDAP between the two groups, with a higher propensity for infection with Gram-positive bacteria, especially CNS in the DM group. One plausible reason for greater number of peritonitis episodes caused by CNS in diabetic population is due to higher risk of touch contamination and incorrect operation of peritoneal fluid exchange consequent to impaired vision due to diabetic retinopathy. The identification of an increased CNS peritonitis rate in this population suggests that more extensive training and more frequent review of operations might be beneficial. In addition, Staphylococcus epidermidis is the most common CNS, which can cause disease under certain circumstances. A study showed that the Staphylococcus epidermidis causing PDAP had low immunogenicity, which makes it more easily establish an infection since it cannot be immediately recognized by the immune system[20]. Meanwhile, DM is related to impaired immunity[21]. We consequently infer that CNS is inclined to colonize in PD patients with DM. Moreover, DM patients are more susceptible to infection especially in poorly controlled diabetics[22]. The impairment of neutrophil oxidative burst in individuals with poorly controlled diabetics may explain this phenomenon. A negative correlation was observed between neutrophil oxidative burst and hemoglobin A1c levels in the study by Osar *et al*[23]. And reduced neutrophil respiratory burst activity in diabetic patients could be restored to almost normal by blood glucose control[24]. However, our findings contrast with a previously published study, which showed that the development of CNS PDAP was not associated with DM[25]. A possible explanation for this discrepancy may be varying microbiological flora over geographical area and time.

E. coli is one of the most frequent causes of PDAP caused by Gram-negative bacteria. In our study, *E. coli* peritonitis was less common in patients with diabetes
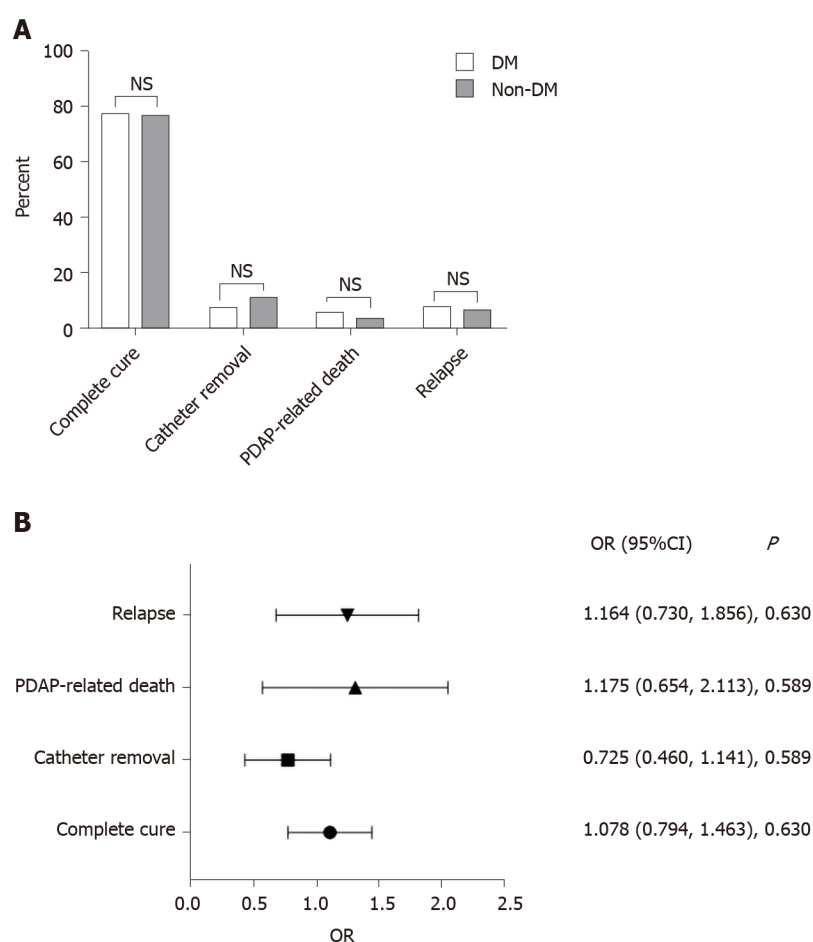
Figure 2 Association between diabetes mellitus and therapeutic outcomes of peritoneal dialysis-associated peritonitis. A: Therapeutic outcomes of peritoneal dialysis-associated peritonitis (PDAP), including complete cure, catheter removal, PDAP-related death, and relapse of PDAP, between the two groups; B: Multivariable logistic regression analysis of relationship between diabetes mellitus and therapeutic outcomes of PDAP. Covariates with $P < 0.05$ in the univariate model and conventional confounders related to therapeutic outcomes (history of diabetes mellitus, age, gender, number of peritonitis episodes, history of cardiovascular diseases, basic hemoglobin, albumin, and estimated glomerular filtration rate) were included in the multivariate regression model. NS: Not significant; PDAP: Peritoneal dialysis-associated peritonitis; DM: Diabetes mellitus; OR: Odds ratio.

with no apparent explanation. Both *E. coli* virulence characteristics and host factors contribute to the development of PDAP. Previous studies showed that the PDAP *E. coli* isolates exhibited a superior virulence capability[26,27]. However, *E. coli* obtained from patients with PDAP did not show a common virulence profile and exhibited diverse serotypes[28]. Difference in virulence patterns of *E. coli* may explain a differential distribution frequency of infection between the DM group and non-DM group.

A noteworthy finding of our study is that the therapeutic outcomes of peritonitis between the DM group and non-DM group are comparable. More importantly, our results were further confirmed by using a multivariable logistic regression model. Our observation had both similarities and differences with previous data from ANZDATA registry study, which included 11122 episodes of peritonitis in 5367 patients in Australia during the period of 2004-2014[29]. Our study demonstrated that the complete cure rates were comparable between DM group and non-DM group, which was similar to the study by Htay *et al*[29]. DM was not associated with PDAP-related death in our study. In contrast, Htay *et al*[29] showed that DM correlated with PDAP-related death. Regional differences may account for this discrepancy.

The effect of DM on long-term prognostic outcomes of PDAP is controversial. A previous study found that DM is a risk factor for all-cause death[13]. Additionally, the study by Tsai *et al*[14] in a single Taiwan center also found a positive relation between DM and PDAP treatment failure, which was defined as death or catheter removal. However, in another study involving 483 patients (69 DM patients) diagnosed with PDAP, patients with DM had similar patient survival with those without DM[11]. In our study, we found a significantly higher all-cause mortality rate in the DM group than in the non-DM group. Higher burden of CVD could explain greater all-cause mortality in patients with diabetes. Patients with diabetes also had a lower level of serum albumin,
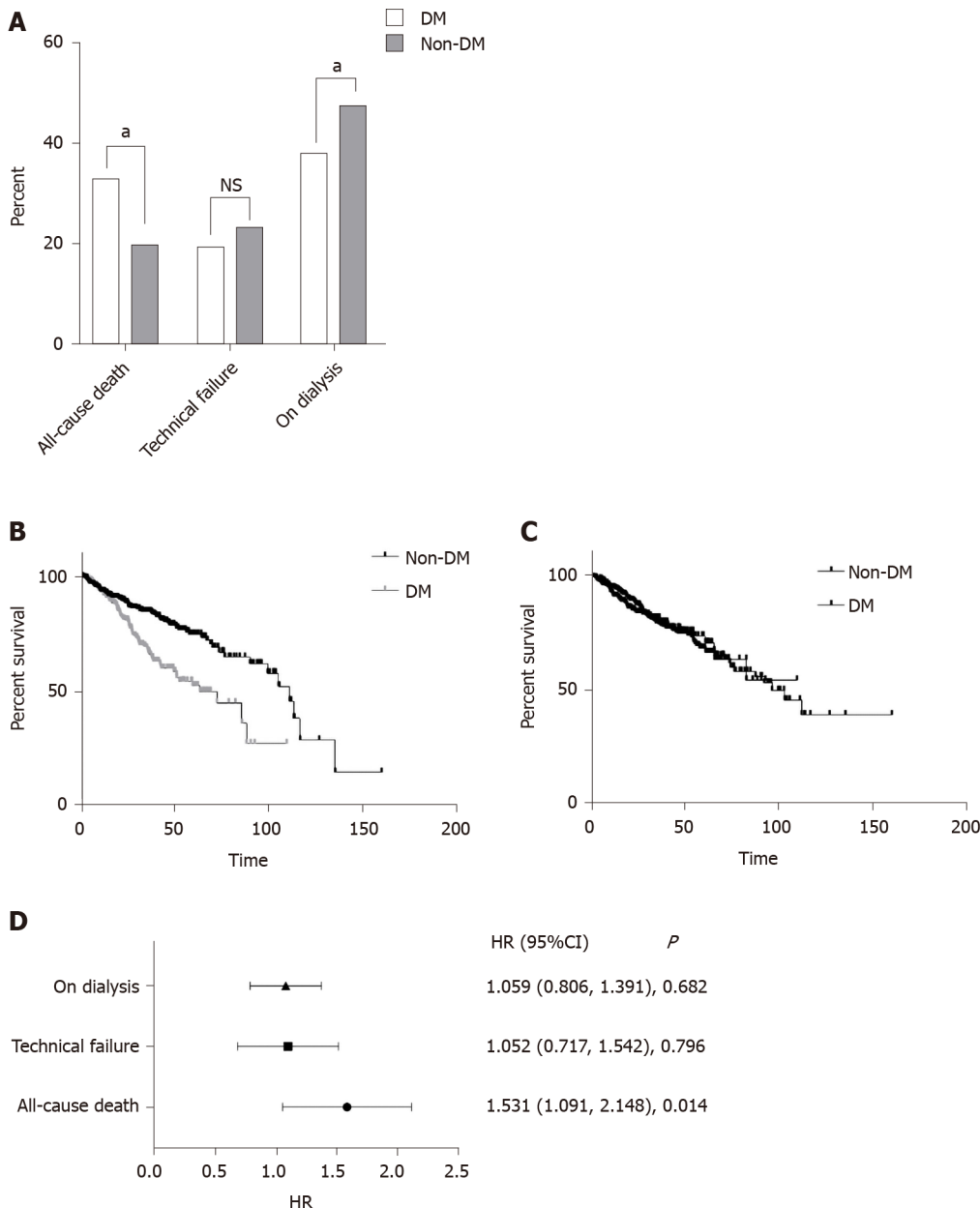
Figure 3 Association between diabetes mellitus and long-term prognostic outcomes of peritoneal dialysis-associated peritonitis. A: Long-term prognostic outcomes of PDAP, including all-cause death, technique failure, and on dialysis between the two groups; B and C: Kaplan-Meier analysis of cumulative patient survival and technique survival according to diabetes mellitus (DM); D: Cox regression analysis of relationship between DM and long-term prognostic outcomes. Covariates with *P* < 0.05 in the univariate model and conventional confounders related to long-term prognostic outcomes (history of DM, age, gender, times of peritonitis episodes, history of cardiovascular diseases, basic hemoglobin, albumin, and estimated glomerular filtration rate) were included in the multivariate regression model. ^a*P* < 0.05, compared between DM group and non-DM group. DM: Diabetes mellitus; HR: Hazard ratio.

which again has been associated with higher mortality in some studies[30,31]. Blood albumin is a marker of both ongoing inflammatory response and malnutrition, which is contained in the malnutrition inflammation score. High malnutrition inflammation score indicates malnourished status in patients undergoing PD[32], which further leads to bad clinical outcomes[33]. Clinicians need to pay more attention to the serum albumin status of patients with diabetes to improve the prognosis of PDAP. The discrepancy between our study and previous studies may be explained by distinct patient characteristics and different covariates included in the Cox proportional hazards model. Similar to the previous study, we also did not find diabetes to be a risk factor for technical failure, further affirming that diabetes should not be considered a hurdle for instituting peritoneal dialysis[11-13].

However, the present study has some limitations. Since it is a retrospective cohort study, potential bias and other confounding factors cannot be entirely excluded. Moreover, we did not consider the effect of indicators such as glycosylated

hemoglobin and fasting blood-glucose on the outcomes of the study.

CONCLUSION

In conclusion, PDAP patients with DM have similar symptomology and are predisposed to CNS but not *E. coli* infection compared those without. DM is not associated with adverse therapeutic outcomes of PDAP. DM is associated with higher all-cause mortality but not technical failure in patients with PDAP.

ARTICLE HIGHLIGHTS

Research background

The number of end-stage renal disease patients with diabetes mellitus (DM) who are undergoing peritoneal dialysis is increasing. Peritoneal dialysis-associated peritonitis (PDAP) is a serious complication of peritoneal dialysis leading to technical failure and increasing mortality in patients undergoing peritoneal dialysis. The profile of clinical symptoms, distribution of pathogenic organisms, and response of PDAP to medical management in the subset of end-stage renal disease patients with DM has not been reported previously. Discrepant results have been found in long-term prognostic outcomes of PDAP in patients with DM. It is important to clarify the clinical features and outcomes of PDAP patients with DM.

Research motivation

PDAP in DM patients is very common in the clinical practice, and treatment of PDAP in DM population is difficult and often with poor prognosis. Our research aimed to study the clinical manifestations, distribution of pathogenic organisms, and outcomes of PDAP in DM patients to provide a basis for future research of reasonable treatment and improvement of prognosis in this population.

Research objectives

This study aimed to compare the clinical features and outcomes of PDAP between patients with DM and those without. We found that the distribution of pathogenic organisms of PDAP was different between the DM group and non-DM group, and DM was a significant predictor of all-cause mortality but not technical failure.

Research methods

This is a multicenter retrospective cohort study. We enrolled patients who had at least one episode of PDAP during the study period. The patients were divided into a DM group and a non-DM group. Clinical features, therapeutic outcomes, and long-term prognostic outcomes were compared between the two groups. Risk factors associated with therapeutic outcomes of PDAP were analyzed using multivariable logistic regression. A Cox proportional hazards model was constructed to examine the influence of DM on patient survival.

Research results

We confirmed that the symptoms of PDAP in the DM group were the same as those of the non-DM group ($P > 0.05$). The DM group had more infections with coagulase-negative Staphylococcus and less infections with *Escherichia coli* (*E. coli*) as compared to the non-DM group. DM was not associated with therapeutic outcomes (complete cure, catheter removal, PDAP-related death, or relapse) of PDAP ($P > 0.05$). The presence of DM was a significant predictor of all-cause mortality (hazard ratio = 1.531, 95% confidence interval: 1.091-2.148, $P < 0.05$), but did not predict occurrence of technical failure ($P > 0.05$). However, we did not consider the effect of indicators such as glycosylated hemoglobin and fasting blood-glucose on the outcomes of the study.

Research conclusions

The symptoms of PDAP are similar in the DM group and non-DM group. Patients with diabetes are predisposed to coagulase-negative Staphylococcus but not *E. coli* infection. DM is associated with higher all-cause mortality but not therapeutic outcomes of PDAP.

Research perspectives

Future research should focus on the effects of blood glucose control on PDAP outcomes, the mechanism of bacterial colonization, and ways to improve prognosis of PDAP in DM patients.

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REFERENCES

1   **Alicic RZ**, Rooney MT, Tuttle KR. Diabetic Kidney Disease: Challenges, Progress, and Possibilities. *Clin J Am Soc Nephrol* 2017; **12**: 2032-2045 [PMID: 28522654 DOI: 10.2215/CJN.11491116]

2   **Tang SCW**, Yu X, Chen HC, Kashihara N, Park HC, Liew A, Goh BL, Nazareth MGC, Bunnag S, Tan J, Lun V, Lydia A, Sharma SK, Hoque E, Togtokh A, Ghnaimet M, Jha V. Dialysis Care and Dialysis Funding in Asia. *Am J Kidney Dis* 2020; **75**: 772-781 [PMID: 31699518 DOI: 10.1053/j.ajkd.2019.08.005]

3   **Coimbra S**, Reis F, Nunes S, Viana S, Valente MJ, Rocha S, Catarino C, Rocha-Pereira P, Bronze-da-Rocha E, Sameiro-Faria M, Oliveira JG, Madureira J, Fernandes JC, Miranda V, Belo L, Santos-Silva A. The Protective Role of Adiponectin for Lipoproteins in End-Stage Renal Disease Patients: Relationship with Diabetes and Body Mass Index. *Oxid Med Cell Longev* 2019; **2019**: 3021785 [PMID: 30911344 DOI: 10.1155/2019/3021785]

4   **de Moraes TP**, Figueiredo AE, de Campos LG, Olandoski M, Barretti P, Pecoits-Filho R; BRAZPD Investigators. Characterization of the BRAZPD II cohort and description of trends in peritoneal dialysis outcome across time periods. *Perit Dial Int* 2014; **34**: 714-723 [PMID: 25185014 DOI: 10.3747/pdi.2013.00282]

5   **Li PK**, Chow KM, Van de Luijtgaarden MW, Johnson DW, Jager KJ, Mehrotra R, Naicker S, Pecoits-Filho R, Yu XQ, Lameire N. Changes in the worldwide epidemiology of peritoneal dialysis. *Nat Rev Nephrol* 2017; **13**: 90-103 [PMID: 28029154 DOI: 10.1038/nrneph.2016.181]

6   **Ghali JR**, Bannister KM, Brown FG, Rosman JB, Wiggins KJ, Johnson DW, McDonald SP. Microbiology and outcomes of peritonitis in Australian peritoneal dialysis patients. *Perit Dial Int* 2011; **31**: 651-662 [PMID: 21719685 DOI: 10.3747/pdi.2010.00131]

7   **Ye H**, Zhou Q, Fan L, Guo Q, Mao H, Huang F, Yu X, Yang X. The impact of peritoneal dialysis-related peritonitis on mortality in peritoneal dialysis patients. *BMC Nephrol* 2017; **18**: 186 [PMID: 28583107 DOI: 10.1186/s12882-017-0588-4]

8   **Boudville N**, Kemp A, Clayton P, Lim W, Badve SV, Hawley CM, McDonald SP, Wiggins KJ, Bannister KM, Brown FG, Johnson DW. Recent peritonitis associates with mortality among patients treated with peritoneal dialysis. *J Am Soc Nephrol* 2012; **23**: 1398-1405 [PMID: 22626818 DOI: 10.1681/ASN.2011121135]

9   **Ozener C**, Arikan H, Karayaylali I, Utas C, Bozfakioglu S, Akpolat T, Ataman R, Ersoy F, Camsari T, Yavuz M, Akcicek F, Yilmaz ME. The impact of diabetes mellitus on peritoneal dialysis: the Turkey Multicenter Clinic Study. *Ren Fail* 2014; **36**: 149-153 [PMID: 24131086 DOI: 10.3109/0886022X.2013.843275]

10   **Chen HL**, Tarng DC, Huang LH. Risk factors associated with outcomes of peritoneal dialysis in Taiwan: An analysis using a competing risk model. *Medicine (Baltimore)* 2019; **98**: e14385 [PMID: 30732176 DOI: 10.1097/MD.0000000000014385]

11   **Tian Y**, Xie X, Xiang S, Yang X, Lin J, Zhang X, Shou Z, Chen J. Risk Factors and Outcomes of Early-Onset Peritonitis in Chinese Peritoneal Dialysis Patients. *Kidney Blood Press Res* 2017; **42**: 1266-1276 [PMID: 29248923 DOI: 10.1159/000485930]

12   **Ma X**, Shi Y, Tao M, Jiang X, Wang Y, Zang X, Fang L, Jiang W, Du L, Jin D, Zhuang S, Liu N. Analysis of risk factors and outcome in peritoneal dialysis patients with early-onset peritonitis: a multicentre, retrospective cohort study. *BMJ Open* 2020; **10**: e029949 [PMID: 32060152 DOI: 10.1136/bmjopen-2019-029949]

13   **TekkariÅŸmaz N**, Torun D. Long-term clinical outcomes of peritoneal dialysis patients: 9-year experience of a single centre in Turkey. *Turk J Med Sci* 2020; **50**: 386-397 [PMID: 32041385 DOI: 10.3906/sag-1909-98]

14   **Tsai CC**, Lee JJ, Liu TP, Ko WC, Wu CJ, Pan CF, Cheng SP. Effects of age and diabetes mellitus on clinical outcomes in patients with peritoneal dialysis-related peritonitis. *Surg Infect (Larchmt)* 2013; **14**: 540-546 [PMID: 24116738 DOI: 10.1089/sur.2012.195]

15   **American Diabetes Association**. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2014; **37** Suppl 1: S81-S90 [PMID: 24357215 DOI: 10.2337/dc14-S081]

16   **Li PK**, Szeto CC, Piraino B, de Arteaga J, Fan S, Figueiredo AE, Fish DN, Goffin E, Kim YL, Salzer W, Struijk DG, Teitelbaum I, Johnson DW. ISPD Peritonitis Recommendations: 2016 Update on Prevention and Treatment. *Perit Dial Int* 2016; **36**: 481-508 [PMID: 27282851 DOI: 10.3747/pdi.2016.00078]

17   **Szeto CC**, Kwan BC, Chow KM, Lau MF, Law MC, Chung KY, Leung CB, Li PK. Coagulase negative staphylococcal peritonitis in peritoneal dialysis patients: review of 232 consecutive cases. *Clin J Am Soc Nephrol* 2008; **3**: 91-97 [PMID: 18032790 DOI: 10.2215/CJN.03070707]

18   **Prasad N**, Sinha A, Gupta A, Sharma RK, Bhadauria D, Chandra A, Prasad KN, Kaul A. Effect of body mass index on outcomes of peritoneal dialysis patients in India. *Perit Dial Int* 2014; **34**: 399-408 [PMID:

24584600 DOI: 10.3747/pdi.2013.00056]

19   **Wu H**, Ye H, Huang R, Yi C, Wu J, Yu X, Yang X. Incidence and risk factors of peritoneal dialysis-related peritonitis in elderly patients: A retrospective clinical study. *Perit Dial Int* 2020; **40**: 26-33 [PMID: 32063144 DOI: 10.1177/0896860819879868]

20   **Jung K**, Lüthje P, Lundahl J, Brauner A. Low immunogenicity allows Staphylococcus epidermidis to cause PD peritonitis. *Perit Dial Int* 2011; **31**: 672-678 [PMID: 20448241 DOI: 10.3747/pdi.2009.00150]

21   **Muller LM**, Gorter KJ, Hak E, Goudzwaard WL, Schellevis FG, Hoepelman AI, Rutten GE. Increased risk of common infections in patients with type 1 and type 2 diabetes mellitus. *Clin Infect Dis* 2005; **41**: 281-288 [PMID: 16007521 DOI: 10.1086/431587]

22   **Rayfield EJ**, Ault MJ, Keusch GT, Brothers MJ, Nechemias C, Smith H. Infection and diabetes: the case for glucose control. *Am J Med* 1982; **72**: 439-450 [PMID: 7036735 DOI: 10.1016/0002-9343(82)90511-3]

23   **Osar Z**, Samanci T, Demirel GY, Damci T, Ilkova H. Nicotinamide effects oxidative burst activity of neutrophils in patients with poorly controlled type 2 diabetes mellitus. *Exp Diabesity Res* 2004; **5**: 155-162 [PMID: 15203886 DOI: 10.1080/15438600490424244]

24   **Ihm SH**, Yoo HJ, Park SW, Park CJ. Effect of tolrestat, an aldose reductase inhibitor, on neutrophil respiratory burst activity in diabetic patients. *Metabolism* 1997; **46**: 634-638 [PMID: 9186297 DOI: 10.1016/s0026-0495(97)90005-6]

25   **Fahim M**, Hawley CM, McDonald SP, Brown FG, Rosman JB, Wiggins KJ, Bannister KM, Johnson DW. Coagulase-negative staphylococcal peritonitis in Australian peritoneal dialysis patients: predictors, treatment and outcomes in 936 cases. *Nephrol Dial Transplant* 2010; **25**: 3386-3392 [PMID: 20466663 DOI: 10.1093/ndt/gfq222]

26   **Li YF**, Su N, Chen SY, Hu WX, Li FF, Jiang ZP, Yu XQ. Genetic background of Escherichia coli isolates from peritoneal dialysis patients with peritonitis and uninfected control subjects. *Genet Mol Res* 2016; **15** [PMID: 27051010 DOI: 10.4238/gmr.15017341]

27   **Lin WH**, Tseng CC, Wu AB, Chang YT, Kuo TH, Chao JY, Wang MC, Wu JJ. Clinical and microbiological characteristics of peritoneal dialysis-related peritonitis caused by Escherichia coli in southern Taiwan. *Eur J Clin Microbiol Infect Dis* 2018; **37**: 1699-1707 [PMID: 29931659 DOI: 10.1007/s10096-018-3302-y]

28   **Dias RCB**, Vieira MA, Moro AC, Ribolli DFM, Monteiro ACM, Camargo CH, Tiba-Casas MR, Soares FB, Dos Santos LF, Montelli AC, da Cunha MLRS, Barretti P, Hernandes RT. Characterization of *Escherichia coli* obtained from patients undergoing peritoneal dialysis and diagnosed with peritonitis in a Brazilian centre. *J Med Microbiol* 2019; **68**: 1330-1340 [PMID: 31347999 DOI: 10.1099/jmm.0.001043]

29   **Htay H**, Cho Y, Pascoe EM, Hawley C, Clayton PA, Borlace M, Badve SV, Sud K, Boudville N, Chen JH, Sypek M, Johnson DW. Multicentre registry data analysis comparing outcomes of culture-negative peritonitis and different subtypes of culture-positive peritonitis in peritoneal dialysis patients. *Perit Dial Int* 2020; **40**: 47-56 [PMID: 32063153 DOI: 10.1177/0896860819879891]

30   **Jiang J**, Wang LH, Fei YY, Zhou XW, Peng L, Lan L, Ren W. Serum Albumin at Start of Peritoneal Dialysis Predicts Long-Term Outcomes in Anhui Han Patients on Continuous Ambulatory Peritoneal Dialysis: A Retrospective Cohort Study. *Kidney Dis (Basel)* 2018; **4**: 262-268 [PMID: 30574503 DOI: 10.1159/000492426]

31   **Matsui M**, Akai Y, Samejima KI, Tsushima H, Tanabe K, Morimoto K, Tagawa M, Saito Y. Prognostic Value of Predialysis Indices for Technique Failure and Mortality in Peritoneal Dialysis Patients. *Ther Apher Dial* 2017; **21**: 493-499 [PMID: 28508538 DOI: 10.1111/1744-9987.12546]

32   **Harvinder GS**, Swee WC, Karupaiah T, Sahathevan S, Chinna K, Ahmad G, Bavanandan S, Goh BL. Dialysis Malnutrition and Malnutrition Inflammation Scores: screening tools for prediction of dialysis-related protein-energy wasting in Malaysia. *Asia Pac J Clin Nutr* 2016; **25**: 26-33 [PMID: 26965758 DOI: 10.6133/apjcn.2016.25.1.01]

33   **Choi HY**, Lee JE, Han SH, Yoo TH, Kim BS, Park HC, Kang SW, Choi KH, Ha SK, Lee HY, Han DS. Association of inflammation and protein-energy wasting with endothelial dysfunction in peritoneal dialysis patients. *Nephrol Dial Transplant* 2010; **25**: 1266-1271 [PMID: 19926717 DOI: 10.1093/ndt/gfp598]

Retrospective Study

Risk of anemia in morbidly obese patients after bariatric surgery in Taiwan

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Abstract

BACKGROUND
Bariatric surgery is one of most effective long-term treatments for morbid obesity. However, post-bariatric surgery anemia is identified as a common adverse effect and remains a challenge nowadays.

AIM
To estimate the risk of post-bariatric surgery anemia and to stratify the association between age, gender, and types of surgery.

METHODS
This study is a population-based cohort study. We conducted this nationwide study using claims data from National Health Insurance Research Database in Taiwan. There were 4373 morbidly obese patients in this study cohort.

RESULTS

Among patients who were diagnosed with morbid obesity, 2864 received bariatric surgery. All obesity-associated comorbidities decreased in the surgical group. Increasing risk of post-bariatric surgery anemia among obese patients was found by Cox proportional hazards regression [adjusted hazard ratio (HR): 2.36]. Also, we found significantly increasing cumulative incidence rate of anemia among patients receiving bariatric surgery by log-rank test. After adjusting for age and gender, the increasing incidence of post-bariatric surgery anemia was found among women (adjusted HR: 2.48), patients in the 20–29-year-old group (adjusted HR: 3.83), and patients in the 30-64-year-old group (adjusted HR: 2.37). Moreover, malabsorptive and restrictive procedures had significantly higher adjusted HRs, 3.18 and 1.55, respectively.

CONCLUSION

Bariatric surgery give rise to anemia risk among obese patients, specifically in women, young- and middle-aged patients, and patients undergoing malabsorptive procedures in our population-based cohort study in Taiwan.

Key Words: Anemia; Bariatric surgery; Malabsorptive procedure; Obesity; Restrictive procedure; Women

Core Tip: Based on a population-based cohort study in Taiwan, this study demonstrated that obese patients receiving bariatric surgery had significantly higher risk of anemia than patients who did not receive bariatric surgery. After adjusting for gender and age, women, young-aged (20-29 years) and middle-aged (30-64 years), had significantly higher incidence of post-bariatric surgery anemia. Both malabsorptive procedures and restrictive procedures increased the incidence of anemia. However, malabsorptive procedures had a higher hazard ratio of post-bariatric surgery anemia than restrictive procedures.

INTRODUCTION

The incidence of obesity has increased rapidly and has tripled over the past decades[1], significantly threatening public health. Bariatric surgery has been shown to be the most effective long-term treatment for morbidly obese patients[1-4] considering that this surgery results in significant and sustainable weight loss and improves comorbidities, long-term mortality, and patients' quality of life[1-8]. Although bariatric surgery is considered safe and has beneficial effects, the development of anemia after bariatric surgery remains a concern. Anemia due to micronutrient deficiencies is identified as a common adverse effect, specifically among patients without regular nutrient supplementation postoperatively.

Post-bariatric surgery anemia can influence as many as two-thirds of patients undergoing bariatric surgery[9]. During these years, several efforts were made to decrease the incidence rate of post-bariatric surgery anemia. First, considering the metabolic sequelae of bariatric surgery, lifelong micronutrient supplementation was considered mandatory[10]. Moreover, the quality and sustainability of medical follow-up consultation became important issues[4]. Some studies revealed that the incidence of anemia is lower in sleeve gastrectomy than that in Roux-en-Y gastric bypass[11-13]. Furthermore, a cohort study in France suggested that the increasing popularity of sleeve gastrectomy is another reason why the incidence of anemia has reduced[4,11]. However, recently, the prevalence of post-bariatric surgery anemia is still considered nonnegligible. In France, 5% of patients were diagnosed with anemia after bariatric surgery between 2008 and 2016[11]. Additionally, if a patient did not receive an outpatient follow-up, the prevalence of anemia could even be 57% 10 years after Roux-

en-Y gastric bypass[14].

Postoperative anemia may develop as a result of several factors. First, absorption of folate and iron mainly happens in the proximal jejunum and duodenum. Malabsorptive procedures, like intestinal bypass, may cause deficiencies of folate, iron, and vitamin B12 and lead to anemia[11,15]. Restrictive procedures, like sleeve gastrectomy, may also reduce the intrinsic factor, gastric acid, and food gastric passing time and subsequently reduce the bioavailability and digestion of nutrients[16]. Furthermore, the net effects observed as a result of the adaptation of bariatric surgery may synergistically affect the hemoglobin level. These complicated factors include reduction of inflammation, adaptation of micronutrient absorption, limited meat intake, attenuated energy intake, and menstruation[12,13,17,18].

A large cohort study from France revealed the long-term anemia incidence among patients who receiving a bariatric procedure[11]. Studies assessing the long-term incidence of post-bariatric surgery anemia have not been conducted yet. This study used the nationwide data [Taiwanese National Health Insurance Research Database (NHIRD)] with large sample size. This study aimed to estimate and compare the long-term incidence of anemia between morbidly obese patients who underwent bariatric surgery *vs* patients who did not undergo bariatric surgery and to stratify the association between gender, age, and types of bariatric surgery in morbidly obese patients who received bariatric surgery or not.

MATERIALS AND METHODS

Data sources
This study is a population-based cohort study in which data were obtained from the NHIRD. The National Health Insurance (NHI) provided coverage for approximately 99.2% of the Taiwan population (more than 23.03 million residents). The NHI is managed by the National Health Insurance Administration (NHIA) since 1995.

The National Health Research Institute (NHRI) obtained the identification-encrypted data from NHIA and established the NHIRD. The Longitudinal Health Insurance Database 2000 (LHID2000), which was used in this study, comprised 1 million randomly sampled medical information from the registry of all beneficiaries in 2000. There was no significant difference in age- and gender-distributions between the data in the LHID2000 and the original NHIRD. The diagnosis code of International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) codes were used.

These data of NHIRD were all de-identified by obfuscating the information about patients and medical facilities to ensure privacy. Moreover, data confidentiality is maintained in accordance with the data regulations of NHIA and NHRI. Because the NHIRD comprises de-identified secondary data for research, informed consent from subjects was waived because anonymous data were used. Furthermore, the Institutional Review Board of China Medical University (CMUH104-REC2-115) approved this study.

Study population and outcomes
Patients who were diagnosed with morbid obesity (ICD-9-CM, 278.01) with age ≥ 20 years and < 100 years and received bariatric surgery (ICD-9-CM, 44.99, 44.95, 44.68, 44.39, 44.38, 44.31, 43.89, and 43.82) between study period January 1, 2000 to December 31, 2010 were recruited. Furthermore, we excluded morbidly obese patients who receiving surgery before obesity diagnosed data, patients who received surgery out of period between 2000 to 2010, patients with diagnosed date of anemia before the index date, those aged < 20 years or ≥ 100 years, and those with missing information of gender or age. Patients who were diagnosed with anemia (ICD-9-CM, 280 and 281.0) and patients who were not diagnosed were analyzed. Morbidly obese patients were identified by the ICD-9-CM codes, with at least one diagnosis in admission during the whole study period. The data from the Registry for Catastrophic Illness Patient Database, a subset of the NHIRD, confirmed the diagnostic accuracy of morbid obesity[19,20].

Potential confounders
By referring to the ICD-9-CM codes, the potential confounding factors for morbid obesity were systematically identified among the data from NHIRD. Age, gender, comorbidities, insurance premium, occupation, medications, and level of urbanization were identified as confounding factors. Hyperlipidemia (ICD-9-CM, 272), diabetes

mellitus (ICD-9-CM, 250, 366.41, 357.2, 362.01-362.02, and 357.2), hypertension (ICD-9-CM, 401–405), coronary artery disease (ICD-9-CM, 411–414), congestive heart failure (ICD-9-CM, 428), stroke (ICD-9-CM, 430–438), asthma (ICD-9-CM, 493), chronic obstructive pulmonary disease (ICD-9-CM, 490–492, 494, and 496), peripheral arterial occlusive disease (ICD-9-CM, 440–444), and chronic kidney disease (ICD-9-CM, 285.21, 250.4, 403-404, and 581–588) were found to be the comorbidities associated with major adverse cardiovascular events. We applied multivariate logistic regression with baseline covariates to calculate propensity scores. The baseline characteristics of study (with bariatric surgery) cohort and comparison (without bariatric surgery) cohort were compared. Furthermore, both cohorts were matched by standardized mean differences, calculated as the difference in proportions or means of a variable divided by a pooled estimate of the standard deviation of the variable.

Statistical analyses

Demographic characteristics differences and comorbidities differences between the study cohort (receiving bariatric surgery) and comparison (without surgery) cohort were analyzed. We conducted the chi-squared test for noncontinuous variables and the two-sample *t*-test for continuous variables. Cox proportional hazards regression was performed to calculat the hazard ratios (HRs) with 95% confidence intervals (CIs) for each variable. Differences in the incidence of major adverse cardiovascular events between the study cohort and comparison cohort were estimated using the Kaplan-Meier curves by performing the log-rank test. Statistical Analysis System (SAS) version 9.4 statistical package (SAS Institute Inc., Cary, NC, United States) was used for statistical analyses. The level of significance was set at 0.05.

RESULTS

A total of 4922 adult patients were hospitalized for morbid obesity during the study period between 2000 to 2010. Of these, 3086 patient received bariatric surgery, and 1666 patients did not receive bariatric surgery. To reduce the influence of pre-surgery anemia, our study group was recruited and analyzed after the exclusion of those who were diagnosed anemia before the index date. Finally, 2864 patients who were diagnosed with morbid obesity and received bariatric surgery in the study cohort and 1509 patients who were diagnosed morbid obesity and did not receive bariatric surgery in the comparison cohort were recruited. (Figure 1). There were significantly more female patients in the study cohort than that in the comparison cohort (64.8% *vs* 54.2%, respectively, *P* < 0.0001) (Table 1). The mean age of the study cohort was significant younger than that of the comparison cohort (33.1 ± 9.1 *vs* 44.3 ± 15.3, respectively, *P* < 0.0001). Compared to the comparison cohort, some demographic characteristics, like hypertension, hyperlipidemia, coronary artery disease, congestive heart failure, stroke, peripheral arterial occlusive disease, chronic kidney disease, chronic obstructive pulmonary disease, diabetes mellitus, gastrointestinal ulcer, and gastrointestinal hemorrhage were significantly lower (*P* < 0.0001) in the study cohort. The means (median) of the follow-up period were 5.68 (5.31) years for the study cohort and 4.21 (3.84) years for the comparison cohort.

In Table 2, asthma, female sex, and gastrointestinal ulcer increased the risk of anemia significantly according to the univariate analyses. Receiving bariatric surgery increased the rate of anemia significantly (HR = 1.71; 95%CI: 1.2-2.44; *P* = 0.003). Receiving bariatric surgery also caused a significantly increased rate of anemia after adjusting for the potential confounding factors of gender, age, and all comorbidities in multivariate analyses (adjusted HR = 2.36; 95%CI: 1.52-3.65; *P* = 0.0001).

The Kaplan-Meier curves of the both cohorts suggested significantly increasing cumulative incidence of anemia in the study cohort (with bariatric surgery) (*P* = 0.0002) (Figure 2).

In Table 3, the HR of post-bariatric surgery anemia and the incidence rate of anemia were stratified by age and gender. Female, age of 20-29 and 30-64 years had significantly higher adjusted HRs (3.83 and 2.87, respectively).

In Table 4, HR of post-bariatric surgery anemia and the incidence rate were stratified by malabsorptive and restrictive procedures. Malabsorptive and restrictive procedures had significantly higher adjusted HRs (3.18 and 1.55, respectively).

Table 1 Demographic characteristics of patients diagnosed morbid obesity receiving and not-receiving bariatric surgery

| Variable | Receiving bariatric surgery | | | | P value[1] |
| | No [*n* = 1509 (34.51%)] | | Yes [*n* = 2864 (65.49%)] | | |
	n	%	*n*	%	
Malabsorptive procedures	-	-	1773	61.91	-
Restrictive procedures	-	-	2465	86.07	-
Sex					< 0.0001
Female	818	54.21	1855	64.77	
Male	691	45.79	1009	35.23	
Age at baseline, yr					< 0.0001
20-29	302	20.01	1305	45.57	
30-64	1034	68.52	1558	54.4	
65-100	173	11.46	1	0.03	
mean ± SD	44.30 ± 15.25		33.09 ± 9.07		< 0.0001[2]
Comorbidities					
Hypertension	751	49.77	254	8.87	< 0.0001
Hyperlipidemia	314	20.81	110	3.84	< 0.0001
Diabetes mellitus	525	34.79	174	6.08	< 0.0001
Coronary artery disease	261	17.3	69	2.41	< 0.0001
Congestive heart failure	243	16.1	39	1.36	< 0.0001
Stroke	123	8.15	37	1.29	< 0.0001
Chronic kidney disease	124	8.22	22	0.77	< 0.0001
Asthma	186	12.33	75	2.62	< 0.0001
Chronic obstructive pulmonary disease	145	9.61	34	1.19	< 0.0001
Peripheral arterial occlusive disease	29	1.92	3	0.1	< 0.0001[a]
Gastrointestinal ulcer	185	12.26	73	2.55	< 0.0001
Gastrointestinal bleeding	44	2.92	12	0.42	< 0.0001

[1]Chi-square test.

[2]*t* test.

[a]Fisher's exact test. The means (median) of follow-up period were 5.68 (5.31) years and 4.21 (3.84) years for the study bariatric surgery cohort and comparison cohorts, respectively. SD: Standard deviation.

DISCUSSION

Our study demonstrated that patients who were diagnosed with morbid obesity and received bariatric surgery had significantly higher risk of anemia than patients who did not receive bariatric surgery. After stratification by gender and age, female sex, young-aged (20-29 years) and middle-aged (30-64 years) patients had significantly higher HRs than male sex and older-aged patients. Malabsorptive procedures had a higher HR of post-bariatric surgery anemia than restrictive procedures.

Currently, bariatric surgery is considered a promising treatment strategy because of its long-term benefits for morbidly obese patients. These benefits include the following: Sustainable body weight loss; improvement in comorbidities; reduction in medicine use; and improvement in patients' quality of life[1-8]. However, post-bariatric surgery anemia, which mainly results from micronutrient deficiencies, is recognized as the most common adverse effect[3-5]. The metabolic sequela, such as anemia, was identified before the 1990s[21,22]. Moreover, recently, post-bariatric surgery anemia remains a challenge. The large cohort study from France revealed that 5% were diagnosed with anemia after bariatric surgery. Furthermore, the overall risk rate of diagnosing anemia postoperatively was 7.8%[11]. In our study, we demonstrate that

Table 2 Cox model with hazard ratio and 95% confidence intervals of anemia associated with receiving bariatric surgery among morbidly obese patients

Variable	Anemia	Crude[1]			Adjusted[2]		
	No. (*n* = 221)	HR	(95%CI)	*P* value	HR	(95%CI)	*P* value
Receiving bariatric surgery							
No	38	1.00	reference		1.00	reference	
Yes	183	1.71	(1.2-2.44)	0.003	2.36	(1.52-3.65)	0.0001
Gender							
Female	186	1.00	reference		1.00	reference	
Male	35	0.32	(0.22-0.46)	< 0.0001	0.33	(0.23-0.48)	< 0.0001
Age							
20-29 years	88	1.00	reference		1.00	reference	
30-64 years	126	1.08	(0.82-1.41)	0.6028	1.06	(0.79-1.41)	0.6956
65-100 years	7	1.33	(0.61-2.88)	0.4724	1.33	(0.54-3.3)	0.5391
Comorbidities (ref = non-)							
Hypertension	41	1.07	(0.76-1.51)	0.6837	1.43	(0.9-2.27)	0.1349
Hyperlipidemia	18	1.06	(0.65-1.71)	0.8249	1.14	(0.64-2.01)	0.6595
Diabetes mellitus	26	0.95	(0.63-1.44)	0.812	0.96	(0.59-1.57)	0.8826
Coronary artery disease	15	1.23	(0.73-2.08)	0.441	1.17	(0.61-2.25)	0.6403
Congestive heart failure	10	1.02	(0.54-1.93)	0.9436	0.91	(0.42-1.94)	0.8027
Stroke	4	0.63	(0.23-1.7)	0.3611	0.52	(0.18-1.5)	0.2286
Chronic kidney disease	7	1.32	(0.62-2.8)	0.4752	1.47	(0.66-3.28)	0.3504
Asthma	17	1.74	(1.06-2.85)	0.0293	1.85	(1.05-3.25)	0.034
Chronic obstructive pulmonary disease	6	0.93	(0.41-2.08)	0.8514	0.56	(0.22-1.43)	0.2267
Peripheral arterial occlusive disease	1	0.99	(0.14-7.05)	0.9905	1.47	(0.2-10.88)	0.705
Gastrointestinal ulcer	20	2.17	(1.37-3.44)	0.001	2.25	(1.34-3.77)	0.0021
Gastrointestinal bleeding	5	2.64	(1.09-6.42)	0.0321	1.83	(0.7-4.78)	0.2204

[1]Crude HR: Represented relative hazard ratio.
[2]Adjusted HR: Represented adjusted hazard ratio: Mutually adjusted for sex, age, receiving bariatric surgery, and baseline comorbidities (as like tables) in Cox proportional hazard regression.

obese patients who received bariatric surgery significantly had 2.36-fold higher risk of anemia than obese patients who did not receive bariatric surgery.

Post-bariatric surgery anemia is considered mainly due to micronutrient deficiency. Deficiencies of vitamin B12, iron, and vitamin D were commonly observed postoperatively[17,23]. Additionally, oral supplementation of these nutrients was described in the updated guidelines[10]. Other beneficial postoperative physiological changes regarding anemia were already determined. Weight loss after bariatric surgery reduces inflammation, consequently reducing anemia. Chronic inflammation has been recognized as a characteristic feature of morbid obesity[24,25]. Decreasing inflammatory markers such as ferritin, C-reactive protein, haptoglobin, and white blood cell count were noted after bariatric surgery[18,26,27]. However, the positive effects of post-bariatric surgery anemia are less observed than the negative effects in the long-term.

This study also proposed that females had a greater risk of post-bariatric surgery anemia than males among obese patients. Women are nearly 3-fold more likely to have a diagnosis of anemia postoperatively compared with men. The results are similar to the result of a study conducted in France[11]. von Drygalski *et al*[18] revealed that Roux-en-Y bypass surgery increased the percentage of anemia from 12% to 23%. During the

Table 3 Incidence rate and hazard ratio with 95% confidence intervals of anemia associated with receiving bariatric surgery, stratified by sex and age

Variable	Receiving bariatric surgery						Receiving *vs* not-receiving bariatric surgery	
	No			Yes			Crude HR	Adjusted HR[2]
	Event	Person years	IR[1]	Event	Person years	IR[1]		
Total	38	6359	5.98	183	16277	11.24	1.71(1.20-2.44)[b]	2.36(1.52-3.65)[c]
Gender								
Female	27	3455	7.82	159	10688	14.88	1.67(1.10-2.53)[a]	2.48(1.50-4.09)[c]
Male	11	2905	3.79	24	5588	4.29	1.24(0.60-2.55)	2.04(0.89-4.70)
Age group, yr								
20-29 yr	4	1386	2.89	84	7825	10.73	3.11(1.14-8.54)[a]	3.83(1.13-12.99)[a]
30-64 yr	27	4341	6.22	99	8446	11.72	1.81(1.18-2.79)[b]	2.37(1.45-3.88)[c]
65-100 yr	7	632	11.07	0	6	0	-	-

[1]IR: Incidence rates, per 1000 person-years.
[2]Represented adjusted hazard ratio: Mutually adjusted for sex, age, receiving bariatric surgery, and baseline comorbidities (as like tables) in Cox proportional hazard regression.
[a]$P < 0.05$.
[b]$P < 0.01$.
[c]$P < 0.001$. HR: Hazard ratio.

Table 4 Cox model with hazard ratio and 95% confidence intervals of anemia associated with receiving malabsorptive procedures or restrictive procedures among morbidly obese patients

Variable	Anemia	Crude[1]			Adjusted[2]		
	No. (*n* = 221)	HR	(95%CI)	*P* value	HR	(95%CI)	*P* value
Malabsorptive procedures							
No	64	1	Reference		1	Reference	
Yes	157	2.76	(2.06-3.70)	< 0.0001	3.18	(2.29-4.40)	< 0.0001
Restrictive procedures							
No	62	1	Reference		1	Reference	
Yes	159	1.38	(1.02-1.86)	0.0356	1.55	(1.10-2.19)	0.0129

[1]Crude HR: Represented relative hazard ratio.
[2]Adjusted HR: Represented adjusted hazard ratio: Mutually adjusted for sex, age, receiving bariatric surgery or not, baseline comorbidities (as like tables) in Cox proportional hazard regression. CI: Confidence interval; HR: Hazard ratio.

postoperative period, premenopausal women have greater prevalence of anemia than postmenopausal women and men. According to the National Health and Nutrition Examination Survey studies, women in their childbearing years generally have a greater percentage of anemia than men (12.2% *vs* 1.5%, respectively), and the gender differences were no longer observed after the age of 50 years[28]. However, data from our study revealed that, after adjusting age and other confounding factors, the risk of post-bariatric surgery anemia is still higher in women than in men. Obesity is associated with an irregular menstruation cycle[29,30]. After bariatric surgery, a more consistent menstrual cycle rather than amenorrhea or irregular menstruation cycle is observed. Hence, premenopausal women are highly diagnosed with anemia postoperatively. Another study suggested that women are at a higher risk of eating less than men, although with limited evidence, which may add other risk for iron deficiency anemia[12].

We also found that young-aged (20-29 years) and middle-aged (30–64 years) patients had significantly higher HRs post-bariatric surgery anemia than older-aged
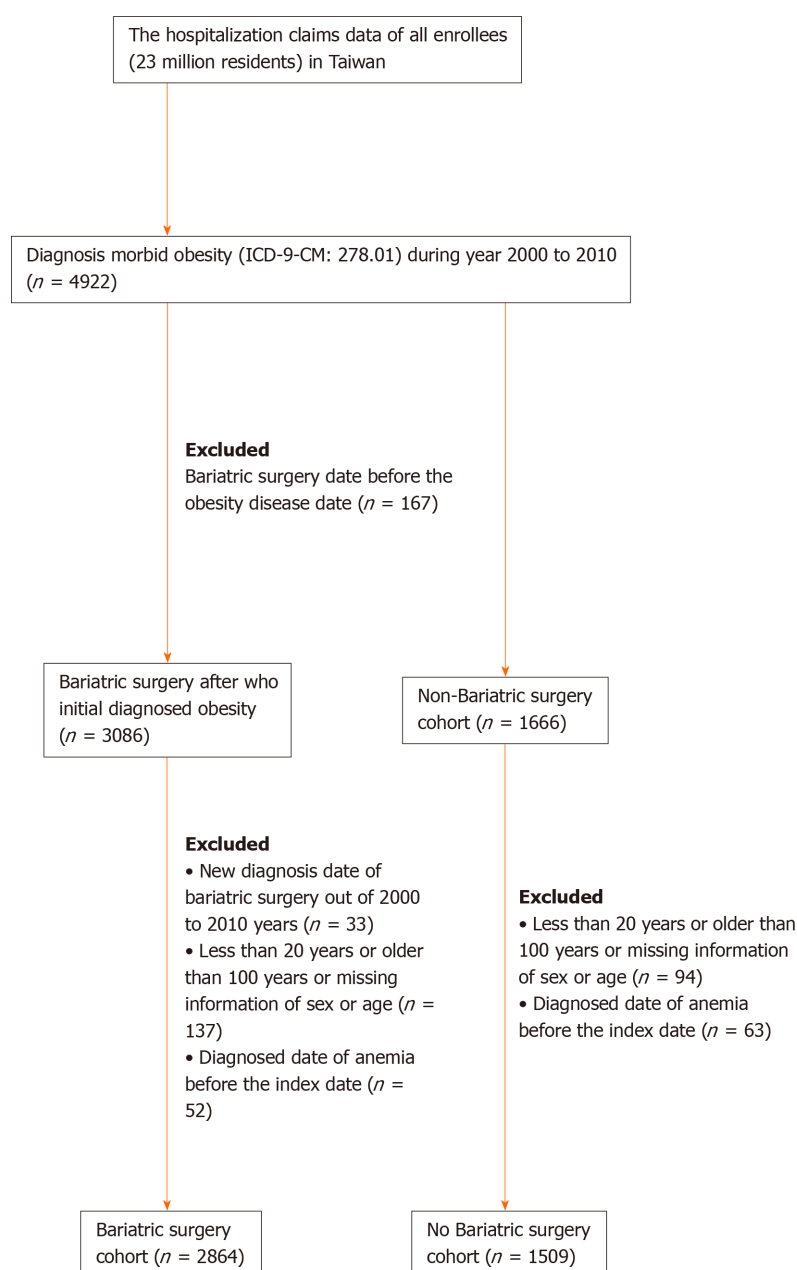
Figure 1 Flowchart of the patient selection (patients diagnosed with morbid obesity who received bariatric surgery) and comparison cohort (patients diagnosed with morbid obesity who did not receive bariatric surgery). ICD-9-CM: International Classification of Diseases, Ninth Revision, Clinical Modification.

patients. A study conducted in France revealed that patients aged less than 52 years have a 50% higher risk of developing post-bariatric surgery anemia than elderly patients[11]. Groups were classified according to the age of 52 years because menstruation was taken into consideration[11]. Thus, considering that premenopausal women are at an increased risk of developing anemia, a French cohort did not consider the effect of age. The following factors may potentially explain the effect of age on anemia after bariatric surgery, although with insufficient evidence: Young-aged individuals consume less calories and nutrients than required and have limited compliance to medical follow-up and limited adherence to micronutrient supplement consumption. Furthermore, the small sample size of older-aged patients, that is, only six older-aged obese patients underwent bariatric surgery, must be taken into consideration.

The risks of post-bariatric surgery anemia vary among different types of surgery. Our study revealed that obese patients who underwent malabsorptive procedures have 3.18-fold higher risk of developing anemia than patients who did not undergo malabsorptive procedures. Moreover, obese patients who underwent restrictive procedures also have 1.55-fold higher risk of developing anemia than patients who did
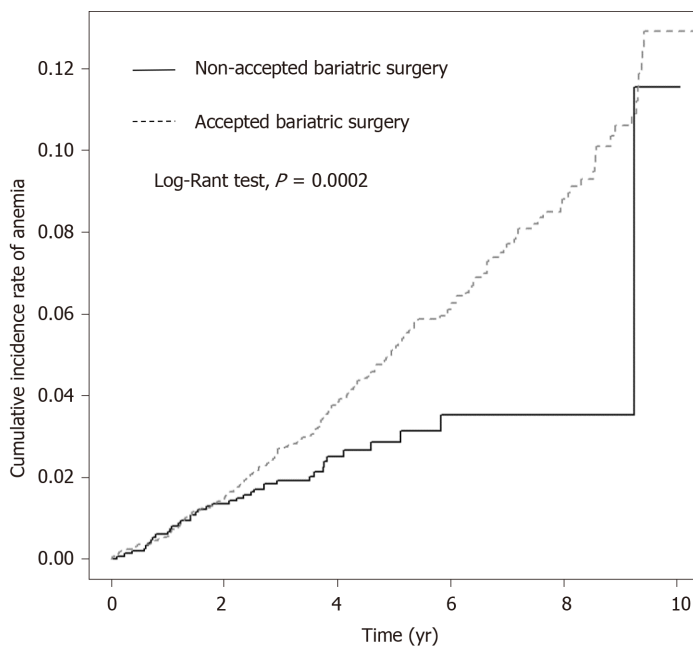
Figure 2 Cumulative incidence of anemia in morbidly obese patients who received bariatric surgery and patients who did not receive bariatric surgery.

not undergo restrictive procedures. Previous studies also demonstrated that malabsorptive procedures have greater risk of anemia than restrictive procedures. The French cohort showed that the risk rates of developing anemia due to micronutrient deficiency were 13.0% after gastric bypass, 5.6% after sleeve gastrectomy, and 4.0% after adjustable gastric banding. Both malabsorptive and restrictive procedures lessen the volume of gastric pouch and also reduce hydrochloric acid production. Gut hypoacidity reduces transit time; consequently, early satiety and decreased intake amount contribute to the risk of micronutrient deficiency anemia after bariatric surgery. In addition to stomach reduction, malabsorptive procedures (*e.g.,* Roux-en-Y bypass surgery) further bypass the main sites of iron absorption (duodenum and a portion of the jejunum). Hence, malabsorptive procedures theoretically result in more iron deficiency. However, patients who underwent malabsorptive procedures are possibly able to adapt iron absorption[5,31]. Therefore, postoperative iron deficiency is insignificantly different between the two types of surgery. One meta-analysis also revealed that sleeve gastrectomy and Roux-en-Y bypass surgery are comparable regarding the risk of postoperative iron deficiency[5].

On the contrary, malabsorptive procedures have significant effects on vitamin B12 absorption. Roux-en-Y bypass surgery reduces acid secretion, affects intrinsic factor function, and limits the mixing of food with pancreatic secretions. Consequently, vitamin B12 maldigestion and malabsorption lead to higher risk anemia postoperatively[5,32]. One meta-analysis revealed that Roux-en-Y bypass surgery had 3.55-fold higher risk of postoperative vitamin B12 deficiency than sleeve gastrectomy[5]. Recent studies demonstrate that malabsorptive procedures result in excess weight loss at midterm, but the difference was not statistically significant. The morbidity rates of the two procedures were statistically insignificant[33,34].

Our study has the following strength: The nationwide cohort with large sample size, paying careful attention to post-bariatric surgery anemia in morbidly obese patients. However, there are some limitations in our study. First, the Taiwan NHIRD did not comprise information regarding hemoglobin level, body weight, and height. Therefore, the associations between body mass index changes, body weight changes, and post-bariatric surgery anemia could not be analyzed. To reduce the influence of pre-surgery anemia, our study group was analyzed after excluding who had anemia diagnosis before index date. Second, due to the retrospective cohort design of the study, the evidence of this study was lower in statistical quality than randomized trials. Third, regimens for micronutrient supplementation were not assessed in this study. Thus, we could not further analyze the micronutrient deficiency between groups.

CONCLUSION

In conclusion, our study demonstrated that morbidly obese patients who received bariatric surgery had a significantly higher risk of developing anemia than patients who did not receive bariatric surgery. After the stratification of confounding factors, female sex, young-aged (20-29 years) and middle-aged (30-64 years) patients, and patients who underwent malabsorptive procedures had significantly higher HRs than male sex, older-aged patients, and patients who did not undergo malabsorptive procedures.

ACKNOWLEDGMENTS

ARTICLE HIGHLIGHTS

Research background

Bariatric surgery is considered to be the most effective long-term treatment for morbidly obese patients. However, post-bariatric surgery anemia is identified as a common adverse effect and remains a challenge nowadays. This study revealed the long-term incidence of anemia in morbidly obese patients who received a bariatric procedure in large cohorts.

Research motivation

Although post-bariatric surgery anemia is identified as a common adverse effect, there are insufficient population-based cohort studies to demonstrate the long-term incidence of anemia and the risk of post-bariatric surgery anemia.

Research objectives

To estimate the risk of post-bariatric surgery anemia and to stratify the association between sex, age, and type of surgery.

Research methods

This study is a population-based cohort study. We conducted this nationwide study using claims data from National Health Insurance Research Database (NHIRD) in Taiwan. There were 4373 morbidly obese patients in this study cohort.

Research results

There were 4373 patients in the cohort. Among patients who were diagnosed with morbid obesity, 2864 received bariatric surgery. All obesity- and obesity-associated comorbidities decreased in the surgical group. Increasing risk of post-bariatric surgery anemia among obese patients was found by Cox proportional hazards regression [adjusted hazard ratio(HR): 2.36]. Also, we found significantly increasing cumulative incidence rate of anemia among patients receiving bariatric surgery by log-rank test. After adjusting for age and sex, the increasing incidence of post-bariatric surgery anemia was found among women (adjusted HR: 2.48), patients in the 20-29-year-old group (adjusted HR: 3.83) and patients in 30–64-year-old group (adjusted HR: 2.37). Moreover, malabsorptive and restrictive procedures had significantly higher adjusted HRs, 3.18 and 1.55, respectively.

Research conclusions

We demonstrated the long-term incidence of post-bariatric surgery anemia and the risk of post-bariatric surgery anemia *via* a population-based cohort study in which data were obtained from the Taiwan NHIRD. Bariatric surgery increases the risk of anemia among obese patients, specifically in women, young- and middle-aged patients, and patients undergoing malabsorptive procedures. Malabsorptive procedures have a higher risk of anemia than restrictive procedures. Bariatric surgery increases the long-term risk of anemia. Considering the risk of post-bariatric surgery anemia, lifelong micronutrient supplementation was considered mandatory. Moreover, the quality and sustainability of medical follow-up consultation became an

important consideration of bariatric surgery.

Research perspectives

A population-based database, like the Taiwan NHIRD, could provide the evidence of long-term risk. The data could also provide information for further analysis of the associated risks. A prospective cohort study or randomized trial could provide better statistical quality.

REFERENCES

1   **Cheng J**, Gao J, Shuai X, Wang G, Tao K. The comprehensive summary of surgical versus non-surgical treatment for obesity: a systematic review and meta-analysis of randomized controlled trials. *Oncotarget* 2016; **7**: 39216-39230 [PMID: 27233078 DOI: 10.18632/oncotarget.9581]

2   **Christou NV**, Sampalis JS, Liberman M, Look D, Auger S, McLean AP, MacLean LD. Surgery decreases long-term mortality, morbidity, and health care use in morbidly obese patients. *Ann Surg* 2004; **240**: 416-23; discussion 423-4 [PMID: 15319713 DOI: 10.1097/01.sla.0000137343.63376.19]

3   **Schauer PR**, Bhatt DL, Kirwan JP, Wolski K, Aminian A, Brethauer SA, Navaneethan SD, Singh RP, Pothier CE, Nissen SE, Kashyap SR; STAMPEDE Investigators. Bariatric Surgery versus Intensive Medical Therapy for Diabetes - 5-Year Outcomes. *N Engl J Med* 2017; **376**: 641-651 [PMID: 28199805 DOI: 10.1056/NEJMoa1600869]

4   **Thereaux J**, Lesuffleur T, Païta M, Czernichow S, Basdevant A, Msika S, Millat B, Fagot-Campagna A. Long-term follow-up after bariatric surgery in a national cohort. *Br J Surg* 2017; **104**: 1362-1371 [PMID: 28657109 DOI: 10.1002/bjs.10557]

5   **Gloy VL**, Briel M, Bhatt DL, Kashyap SR, Schauer PR, Mingrone G, Bucher HC, Nordmann AJ. Bariatric surgery versus non-surgical treatment for obesity: a systematic review and meta-analysis of randomised controlled trials. *BMJ* 2013; **347**: f5934 [PMID: 24149519 DOI: 10.1136/bmj.f5934]

6   **Sjöström L**, Peltonen M, Jacobson P, Sjöström CD, Karason K, Wedel H, Ahlin S, Anveden Å, Bengtsson C, Bergmark G, Bouchard C, Carlsson B, Dahlgren S, Karlsson J, Lindroos AK, Lönroth H, Narbro K, Näslund I, Olbers T, Svensson PA, Carlsson LM. Bariatric surgery and long-term cardiovascular events. *JAMA* 2012; **307**: 56-65 [PMID: 22215166 DOI: 10.1001/jama.2011.1914]

7   **Huang HH**, Lee WJ, Chen SC, Chen TF, Lee SD, Chen CY. Bile Acid and Fibroblast Growth Factor 19 Regulation in Obese Diabetics, and Non-Alcoholic Fatty Liver Disease after Sleeve Gastrectomy. *J Clin Med* 2019; **8**: 815 [PMID: 31181641 DOI: 10.3390/jcm8060815]

8   **Wang W**, Fann CSJ, Yang SH, Chen HH, Chen CY. Weight loss and metabolic improvements in obese patients undergoing gastric banding and gastric banded plication: A comparison. *Nutrition* 2019; **57**: 290-299 [PMID: 30219686 DOI: 10.1016/j.nut.2018.05.024]

9   **Brolin RE**, LaMarca LB, Kenler HA, Cody RP. Malabsorptive gastric bypass in patients with superobesity. *J Gastrointest Surg* 2002; **6**: 195-203; discussion 204-5 [PMID: 11992805 DOI: 10.1016/s1091-255x(01)00022-1]

10   **Parrott J**, Frank L, Rabena R, Craggs-Dino L, Isom KA, Greiman L. American Society for Metabolic and Bariatric Surgery Integrated Health Nutritional Guidelines for the Surgical Weight Loss Patient 2016 Update: Micronutrients. *Surg Obes Relat Dis* 2017; **13**: 727-741 [PMID: 28392254 DOI: 10.1016/j.soard.2016.12.018]

11   **Bailly L**, Schiavo L, Sebastianelli L, Fabre R, Pradier C, Iannelli A. Anemia and Bariatric Surgery: Results of a National French Survey on Administrative Data of 306,298 Consecutive Patients Between 2008 and 2016. *Obes Surg* 2018; **28**: 2313-2320 [PMID: 29516395 DOI: 10.1007/s11695-018-3143-x]

12   **Kheniser KG**, Kashyap SR, Schauer PR, Lam ETC, Kullman ES. Prevalence of Anemia in Subjects Randomized into Roux-en-Y Gastric Bypass or Sleeve Gastrectomy. *Obes Surg* 2017; **27**: 1381-1386 [PMID: 28185151 DOI: 10.1007/s11695-017-2573-1]

13   **Kwon Y**, Kim HJ, Lo Menzo E, Park S, Szomstein S, Rosenthal RJ. Anemia, iron and vitamin B12 deficiencies after sleeve gastrectomy compared to Roux-en-Y gastric bypass: a meta-analysis. *Surg Obes Relat Dis* 2014; **10**: 589-597 [PMID: 24582411 DOI: 10.1016/j.soard.2013.12.005]

14   **Chen GL**, Kubat E, Eisenberg D. Prevalence of Anemia 10 Years After Roux-en-Y Gastric Bypass in a Single Veterans Affairs Medical Center. *JAMA Surg* 2018; **153**: 86-87 [PMID: 28975250 DOI: 10.1001/jamasurg.2017.3158]

15   **Malinowski SS**. Nutritional and metabolic complications of bariatric surgery. *Am J Med Sci* 2006; **331**: 219-225 [PMID: 16617238 DOI: 10.1097/00000441-200604000-00009]

16   **Braghetto I**, Davanzo C, Korn O, Csendes A, Valladares H, Herrera E, Gonzalez P, Papapietro K. Scintigraphic evaluation of gastric emptying in obese patients submitted to sleeve gastrectomy compared to normal subjects. *Obes Surg* 2009; **19**: 1515-1521 [PMID: 19714384 DOI: 10.1007/s11695-009-9954-z]

17   **Chou JJ**, Lee WJ, Almalki O, Chen JC, Tsai PL, Yang SH. Dietary Intake and Weight Changes 5 Years After Laparoscopic Sleeve Gastrectomy. *Obes Surg* 2017; **27**: 3240-3246 [PMID: 28589529 DOI: 10.1007/s11695-017-2765-8]

18   **von Drygalski A**, Andris DA, Nuttleman PR, Jackson S, Klein J, Wallace JR. Anemia after bariatric surgery cannot be explained by iron deficiency alone: results of a large cohort study. *Surg Obes Relat Dis* 2011; **7**: 151-156 [PMID: 20702143 DOI: 10.1016/j.soard.2010.04.008]

19   **Huang HH**, Hsieh MS, Chen CY. Risk of cholecystectomy in morbidly obese patients after bariatric surgery in Taiwan. *Obes Res Clin Pract* 2019; **13**: 191-196 [PMID: 30665822 DOI: 10.1016/j.orcp.2019.01.001]

20   **Chang CM**, Hsieh MS, Yang TC, Hsieh VC, Chiang JH, Huang HH, How CK, Hu SY, Yen DH. Selective serotonin reuptake inhibitors and the risk of hepatocellular carcinoma in hepatitis B virus-infected patients.

Cancer Manag Res 2017; **9**: 709-720 [PMID: 29238221 DOI: 10.2147/CMAR.S148097]

21   **Amaral JF**, Thompson WR, Caldwell MD, Martin HF, Randall HT. Prospective hematologic evaluation of gastric exclusion surgery for morbid obesity. *Ann Surg* 1985; **201**: 186-193 [PMID: 3970598 DOI: 10.1097/00000658-198502000-00009]

22   **Ponsky TA**, Brody F, Pucci E. Alterations in gastrointestinal physiology after Roux-en-Y gastric bypass. *J Am Coll Surg* 2005; **201**: 125-131 [PMID: 15978453 DOI: 10.1016/j.jamcollsurg.2005.03.021]

23   **Gletsu-Miller N**, Wright BN. Mineral malnutrition following bariatric surgery. *Adv Nutr* 2013; **4**: 506-517 [PMID: 24038242 DOI: 10.3945/an.113.004341]

24   **Cottam DR**, Mattar SG, Barinas-Mitchell E, Eid G, Kuller L, Kelley DE, Schauer PR. The chronic inflammatory hypothesis for the morbidity associated with morbid obesity: implications and effects of weight loss. *Obes Surg* 2004; **14**: 589-600 [PMID: 15186624 DOI: 10.1381/096089204323093345]

25   **Chen CY**, Fujimiya M, Laviano A, Chang FY, Lin HC, Lee SD. Modulation of ingestive behavior and gastrointestinal motility by ghrelin in diabetic animals and humans. *J Chin Med Assoc* 2010; **73**: 225-229 [PMID: 20685586 DOI: 10.1016/S1726-4901(10)70048-4]

26   **Agrawal V**, Krause KR, Chengelis DL, Zalesin KC, Rocher LL, McCullough PA. Relation between degree of weight loss after bariatric surgery and reduction in albuminuria and C-reactive protein. *Surg Obes Relat Dis* 2009; **5**: 20-26 [PMID: 18951068 DOI: 10.1016/j.soard.2008.07.011]

27   **Anty R**, Dahman M, Iannelli A, Gual P, Staccini-Myx A, Amor IB, Luciani N, Saint-Paul MC, Huet PM, Sadoul JL, Srai SK, Unwin R, Gugenheim J, Le Marchand-Brustel Y, Tran A, Bekri S. Bariatric surgery can correct iron depletion in morbidly obese women: a link with chronic inflammation. *Obes Surg* 2008; **18**: 709-714 [PMID: 18330662 DOI: 10.1007/s11695-007-9276-y]

28   **Guralnik JM**, Eisenstaedt RS, Ferrucci L, Klein HG, Woodman RC. Prevalence of anemia in persons 65 years and older in the United States: evidence for a high rate of unexplained anemia. *Blood* 2004; **104**: 2263-2268 [PMID: 15238427 DOI: 10.1182/blood-2004-05-1812]

29   **Chang PJ**, Chen PC, Hsieh CJ, Chiu LT. Risk factors on the menstrual cycle of healthy Taiwanese college nursing students. *Aust N Z J Obstet Gynaecol* 2009; **49**: 689-694 [PMID: 20070725 DOI: 10.1111/j.1479-828X.2009.01097.x]

30   **Wei S**, Schmidt MD, Dwyer T, Norman RJ, Venn AJ. Obesity and menstrual irregularity: associations with SHBG, testosterone, and insulin. *Obesity (Silver Spring)* 2009; **17**: 1070-1076 [PMID: 19180069 DOI: 10.1038/oby.2008.641]

31   **Schümann K**, Elsenhans B, Forth W, Schroeder P. Intestinal iron transfer after ileojejunal transposition. *Digestion* 1991; **50**: 182-193 [PMID: 1812044 DOI: 10.1159/000200760]

32   **Smith CD**, Herkes SB, Behrns KE, Fairbanks VF, Kelly KA, Sarr MG. Gastric acid secretion and vitamin B12 absorption after vertical Roux-en-Y gastric bypass for morbid obesity. *Ann Surg* 1993; **218**: 91-96 [PMID: 8328834 DOI: 10.1097/00000658-199307000-00014]

33   **Salminen P**, Helmiö M, Ovaska J, Juuti A, Leivonen M, Peromaa-Haavisto P, Hurme S, Soinio M, Nuutila P, Victorzon M. Effect of Laparoscopic Sleeve Gastrectomy vs Laparoscopic Roux-en-Y Gastric Bypass on Weight Loss at 5 Years Among Patients With Morbid Obesity: The SLEEVEPASS Randomized Clinical Trial. *JAMA* 2018; **319**: 241-254 [PMID: 29340676 DOI: 10.1001/jama.2017.20313]

34   **Shoar S**, Saber AA. Long-term and midterm outcomes of laparoscopic sleeve gastrectomy versus Roux-en-Y gastric bypass: a systematic review and meta-analysis of comparative studies. *Surg Obes Relat Dis* 2017; **13**: 170-180 [PMID: 27720197 DOI: 10.1016/j.soard.2016.08.011]

Impact of technology use in type 2 diabetes distress: A systematic review

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Abstract

BACKGROUND

Diabetes distress is an important factor in treatment outcomes and results in poor behavioral and biological consequences. Technology has been used in management programs of diabetes to improve communication between patients and health care providers and to promote education about the disease and its psychological aspects, which can impact the self-efficacy of the programs. However, the true impact of technological approaches on the management of type 2 diabetes distress remains controversial.

AIM

To investigate the effectiveness of technology interventions on the management of type 2 diabetes distress.

METHODS

Studies published from 2014 to 2019 were searched in five databases: MEDLINE, PubMed, Library and Information Science Source, Academic Search Ultimate and PsycINFO. The Boolean logic search terms were: (1) T2Diabetes; (2) diabetes distress; and (3) technology OR mobile OR phone OR application OR web. We also systematically searched the reference lists of the included studies and relevant reviews. Randomized controlled trials with technology interventions, type 2 diabetes patients and diabetes distress as the outcome were selected. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement was followed.

RESULTS

Of the 88 studies selected, nine full articles met the inclusion criteria and were subjected to final careful review. On the JADAD scale, one article was classified as having poor quality and eight as having good quality. Six out of nine articles showed that technology interventions had a positive impact on diabetes distress scale scores when compared with the initial data. Among the six articles, five showed a greater reduction in the diabetes distress scores from control interventions. Web-based interventions had good results when users received personalized feedback and routine caregiver support and attention.

CONCLUSION

Technology interventions can contribute positively to the management of type 2 diabetes distress, especially with a tailored approach in conjunction with caregiver interaction with patients.

Key Words: Technology; Type 2 diabetes; Diabetes distress; Healthcare; Systematic review

Core Tip: Technology interventions can impact the reduction of diabetes distress and improve the outcome and quality of life of patients with type 2 diabetes mellitus.

INTRODUCTION

Diabetes mellitus is one of the most prevalent diseases in the world. Today, the worldwide incidence of diabetes is estimated to be over 450 million[1]. Type 2 diabetes (T2D) comprises approximately 90% of cases and is associated with modifiable factors, genetics and aging. Target organ lesions such as nephropathy, neuropathy, retinopathy and cardiopathy are long-term results of hyperglycemia. Organ complications and attempts at diabetes control may affect physical and emotional health and patient quality of life, leading to negative psychological conditions. Patients with depressive symptoms present with more hospitalization days, poorer self-management behavior, more absenteeism, and increased morbidity and mortality. However, it is important to highlight that most diabetic patients with high levels of depressive symptoms are not clinically depressed, rather, they could be suffering from diabetic-specific distress consequences[2].

Diabetes distress (DD) is defined as the fears, worries and concerns of individuals with type 1 or type 2 diabetes related to the emotional responses to diagnosis, risk of complications, self-management demands, unresponsive providers and quality of interpersonal relationships. Identifying patients with DD and addressing the social, personal and health-related causes of distress might have a greater impact than prescribing treatments for clinical depression[2]. Initially, to serve as a screening measure for DD, the Problem Areas in Diabetes scale (PAID), a 20-item questionnaire with no subscales, was developed and has been linked to diabetes self-care behaviors and glycemic controls[3,4]. In 2009, McGuire *et al*[5] validated the PAID-5, a short version of PAID with items 3, 6, 12, 16 and 19 of the original scale, with 94% sensitivity and 80% specificity.

In 2005, Polonsky *et al*[3] validated a specific diabetes distress scale (DDS) with a 17-item self-reported questionnaire that captures four critical dimensions of distress: Emotional burden, regimen distress, interpersonal distress and physician distress. Higher DDS scores are associated with poorer diabetes outcomes, such as high HbA1c, low self-efficacy, choosing unhealthy foods[3] and even an increase in coronary artery disease incidence[6].

In 2008, Fisher *et al*[4] presented a two-item screening version of the DDS, with items "feeling overwhelmed by the demands of living with diabetes" and "feeling that I am

often failing with my diabetes regimen", which showed good sensitivity (95%) and specificity (85%).

Simultaneously, in an attempt to measure DD, data management technologies, web-based interventions, telemedicine, mobile phones, applications and others have been used as modern tools of communication to improve healthcare. Some authors have already demonstrated that technological devices could enhance engagement, adherence, cost effectiveness and access to health interventions[7,8], having an impact on blood glucose control and T2D self-management[9-12]. However, review authors have are in disagreement about the benefits of this technology in T2D distress[13,14]. The primary aim of this review is to determine the impact of programs with technological interventions regarding disease management, not just as a communication alternative, on T2D distress through a DDS measurement study.

MATERIALS AND METHODS

Data sources and search strategy

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement and checklist were followed in this study. The review protocol is registered on PROSPERO (https://www.crd.york.ac.uk/PROSPERO/), an international prospective register of systematic reviews with 160386 registered numbers.

The electronic databases used were MEDLINE, PubMed and EBSCO (which includes three databases with duplicates removed automatically: Library and Information Science Source, Academic Search Ultimate and PsycINFO) for studies published in English from January 2014 through December 2019. The Boolean logic search terms were: (1) T2Diabetes; (2) Diabetes distress; and (3) Technology OR mobile OR phone OR application OR web. We also systematically searched the reference lists of the included studies and relevant reviews.

Study selection

Following the removal of duplicates, titles and/or abstracts were screened by the first reviewer and then a second reviewer. If a disagreement occurred, a third reviewer was consulted.

Inclusion and exclusion criteria

Inclusion criteria were studies with: (1) Only subjects over 18-years-old; (2) Subjects with T2D; (3) Randomized controlled trials (RCTs); (4) Any intervention with technology; use and (5) DDS present in main or secondary outcomes.

Exclusion criteria were studies with: (1) Non-English language text; (2) absence of a control group without technological intervention; and (3) the inclusion of only pregnant women.

Data extraction

The investigators collected the following from each eligible study in a full article screening: (1) Number of subjects recruited for randomization, including the presence of sample size calculations; (2) Main demographic descriptions, including age with standard deviation, gender and study design including duration; (3) Description of intervention and control groups; (4) distress outcome measures; and (5) Statistical significance results.

Methodologic quality

The JADAD scale was used to measure the likelihood of bias and was applied to each selected study by the two reviewers independently.

RESULTS

Study selection

A PRISMA flowchart (Figure 1) summarizes the results of the search, screening process and reasons for exclusion. From the database sources, we collected 87 studies: (1) MEDLINE: 18; (2) PubMed: 36; and (3) EBSCO (Library and Information Science Source, Academic Search Ultimate and PsycINFO): 33. One article from the reference lists was included. After 18 duplicates were excluded (EBSCO system automatically
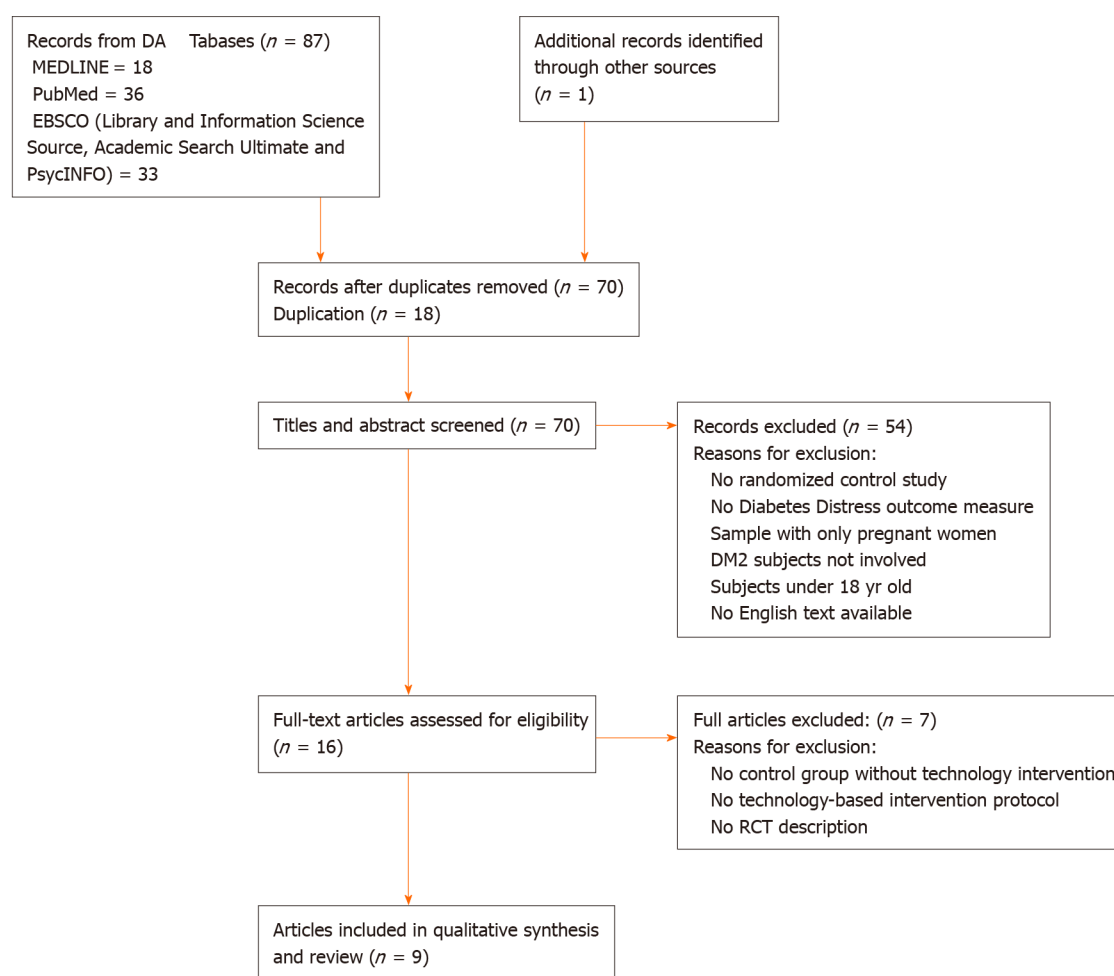
Figure 1 Flow diagram of search results and reasons for exclusion. RCT: Randomized controlled trials.

excluded duplicated references from its three databases) by the two reviewers' analysis, 70 references with titles and abstracts remained for screening. Studies with no randomized control design, no DD outcome measure, no involvement of T2D subjects, no English text available, samples with only pregnant women or individuals less than 18-years-old were excluded. Next, out of the 16 full text articles selected, 7 were excluded due to the absence of a control group without technology-based intervention or for only containing an RCT protocol description; the remaining nine full articles were analyzed for data extraction[15-23] (Figure 1).

Data extraction

Data extraction items are shown in Table 1. Of the nine articles analyzed, two did not present sample size calculations[15,22] including technique description. Three RCTs[17,19,23] used the DDS-17 items for the DD measure, four used the PAID-5[16,18,21,23], one used two subscales of the DDS, the five-item Regimen Distress subscale and the five-item Emotional Burden subscale version[15], and one used the PAID with 20 items[15]. In terms of demographics, female gender was the majority in six articles[15,16,19,20,22,23], and in one, no gender reporting was found[17]. Follow-up varied from 8 to 48 wk. One study had an 8-wk[16] period, one had 10 wk[20], two studies had 12 wk[17,23], three studies had 24 wk[18,21,22] and two had 48 wk[15,19] (Table 1).

Methodologic quality

The quality assessment of the studies with the JADAD scale is presented in Table 2. Eight RCTs were scored with good quality (total score ≥ 3) and low bias risk. One study[22] was scored as poor quality with high bias risk. The main topic with the fewest points was blindness, and three articles had a double-blind design[19,20,23] (Table 2).

Table 1 Data extracted

Ref.	Sample	SSC	Age average	Gender	Duration	IEWT	CGITE	DDS version used and data results	Statistical significance between groups
Fisher *et al*[15], 2014	392	No	56	53.8% Female, 46.2% Male	48 wk	My path to a healthy life computer-assisted self-management plus problem-solving therapyTechnology: Phone calls and web-based diabetes self-management and diabetes distress change program	Leap ahead program delivers diabetes information only, and participants were not directed to use the information to engage in a specific or structured program of self-management or diabetes distress change	DDS (5- item Regimen Distress Subscale and 5-item Emotional Burden Subscale from DDS)	$P = 0.50$, No significant
Nobis *et al*[16], 2015	260	Yes	51	63% Female, 37% Male	8 wk	GET.ON Mood Enhancer personalized, guided, Internet-based diabetes self-help intervention with personalized feedback from psychologist	Control Group: Unguided psychoeducation program	DDS (PAID-5)	$P < 0.001$, Significant
Bajaj *et al*[17], 2016	139	Yes	56.4	NR	12 wk	Long-acting insulin glargine Titration Web Tool (LTHome), instructions on insulin administration and dosing, as well as the use of the web-based LTHome tool (containing a rules engine-based algorithm for titration), provided by a delegated nonhealthcare professional Technology: Web-based insulin titration algorithm embedded in a range of platforms, including glucometer, personal computer and mobile phones	EUT of Glargine Titration: Insulin dosing and titration instructions were provided by CDEs according to a standard protocol	17-item DDS	$P = 0.04$; Significant
Rondags *et al*[18], 2016	137	Yes	52	46% Female, 54% Male	24 wk	HypoAware consists of three group sessions and is combined with two online modules. Group sessions are highly interactive and aimed at patient empowerment to improve symptom recognition, risk awareness, preventive and problem-solving strategies and coping with (the risk of) hypoglycemia Technology: two online modules	Care as usual had access to comprehensive diabetes care as normally provided by their diabetes team	DDS (PAID-5)	$P = 0.365$, No significant
Holland-Carter *et al*[19], 2017	563	Yes	55.1	71% Female, 29% Male	48 wk	WW approach, supplemented with phone and email counseling with a CDE Technology: WW online tools, unlimited phone calls and email diabetes educator consultation	SC, one session of face-to-face T2DM nutritional counseling by a registered dietitian as well as follow-up written information	17-item DDS	$P < 0.001$, Significant
Newby *et al*[20], 2017	106	Yes	47	71% Female, 29% Male	10 wk	ICBT not tailored to diabetes	TAU control group	DDS (PAID 20 items)	$P < 0.001$, Significant
Ebert *et al*[21], 2017	260	Yes	50.8	43.8% Female, 56.2% Male	24 wk	GET.ON Mood Enhancer personalized, guided, Internet-based diabetes self-help intervention with personalized feedback from psychologist Technology: active online training on diabetes and depression, personalized approach	Control: Usual treatment	DDS (PAID-5)	$P < 0.001$, Significant
Schlicker *et al*[22], 2019	253	No	50.7	62.8% Female, 37.2% Male	24 wk	GET.ON Mood Enhancer personalized, guided, Internet-based diabetes self-help intervention with personalized feedback from psychologist	Placebo online, online psychoeducation control condition	DDS (PAID-5)	$P = 0.75$, No significant
Clarke *et al*[23], 2019	780	Yes	58	68.8% Female, 31.2% Male	12 wk	My compass program is a fully automated, web- based cognitive behavioral, self-guided public health treatment program for common mental health problems with a personalized treatment plan based on an assessment of user symptoms. Technology: Web-based, fully automated program with self-guided cognitive behavioral treatment through personal computer or mobile	Healthy lifestyles: Placebo without therapeutic, only informative, no feedback content	17-item DDS	$P = 0.36$, No significant

phone

P values < 0.05 were considered statistically significant. SSC: Sample size calculation; IEWT: Intervention elaborated with technology; CGITE: Control group with no intervention technology elaborated; DDS: Diabetes distress scale; EUT: Enhanced usual therapy; CDE: Certified diabetes educator; WW: Weight watchers; SC: Standard care; T2D: Type 2 diabetes; ICBT: Internet cognitive behavioral therapy; TAU: Treatment as usual; NR: Not related.

DISCUSSION

Main findings

This review suggests that technology could have a positive impact on DD in T2D patients. The majority of articles selected for qualitative synthesis (six[16,17,19,20,23] out of nine[15,18,22]) showed significant DD scale improvement in the technology intervention groups over the initial data. Five articles[16,17,19-21] showed significant differences between groups. Studies with technological interventions had significantly lower DDS scores at the end than at baseline. Although a study did not find significant differences between groups, all participants showed symptom improvement in DDS scores, including the control group.

Rondags *et al*[18] did not find a significant difference in the DDS scores between the groups but described a 30% drop in the HypoAware Group (technology-based intervention) in distress concerning hypoglycemia.

Regarding quality, perhaps as a result of the RCT inclusion criteria, only one article had a high bias risk on the JADAD scale, and two had no sample size calculation, reflecting good scientific quality of the articles reviewed.

Type of technology intervention

Newby *et al*[20] and Clarke *et al*[23] showed different findings for generic web-based interventions with psychological content and highlighted the necessity of a diabetes-specific web-based approach. In contrast, Nobis *et al*[16] and Ebert *et al*[21] found similar results concerning diabetic distress improvement with the same web-based mood enhancer intervention (GET.ON MED).

Web-based interventions had better results when users received program feedback personalized in its content[24]. According to this affirmative, all publications with DDS improvement in our review presented tailored technology interventions with personal adjustments in their programs[16,17,19,20,23]. Thus, it seems that more than a diabetes-tailored approach, patient-tailored and-guided technological programs were more successful in type 2 DDS improvement.

However, these findings are in contrast to the results of Mathiesen *et al*[13] in their trial about the influence of technology interventions on T2D distress. They attributed their findings to the vulnerability of T2D patients facing tailored digital interventions, resulting in an increase in distress, such as "suffering informational confusion, experiencing digital alienation, and missing the human touch", mainly because "navigating a complex digital portal on diabetes might be more challenging than

Table 2 JADAD scale

Ref.	Randomization, method	Double blind	Descriptions of withdrawals and dropouts	Total
Bajaj *et al*[17]	1 + 1	0	1	3
Clarke *et al*[23]	1 + 1	1 + 1	1	5
Ebert *et al*[21]	1 + 1	1	1	4
Fisher *et al*[15]	1 + 1	0	1	3
Holland-Carter *et al*[19]	1 + 1	1 + 1	1	5
Nobis *et al*[16]	1 + 1	0	1	3
Rondags *et al*[18]	1 + 1	0	1	3
Schlicker *et al*[22]	1	0	0	1
Newby *et al*[20]	1 + 1	1 + 1	1	5

simply accessing the site" and the digital caregivers' approach. Despite the limitations of this study, such as the small sample size (12 subjects) and less scientific evidence compared to the nine articles analyzed in the present review, we considered some of the authors' conclusions to improve our considerations. For example, the success of a digital T2D-tailored program depends on the quality of the caregiver-patient relationship, and topics such as digital buddy rights choice and training for vulnerable T2D patient care or a wider social network must be included in the technology T2D intervention framework.

Diabetes distress scale

Possible biases arising from the different scales of DD in the included studies (17-item DDS, PAID and PAID-5) became null due to the use of the same distress scale in the intervention and control groups. This is the main reason to consider only DDS scales in the review and not any of the isolated distress symptoms or other measures in the outcome.

Limitations

First, our review did not discriminate psychological level in article subject recruitment, and some authors attributed differences in the impact of DD approaches to baseline depression levels[20,23], and RCT studies may not be comparable in their demographic composition. However, we selected only RCTs, and the outcome was DDS improvement between groups with the same inclusion and exclusion criteria. That reduces possible biases such as better intervention results for populations with worse baseline levels of depression. Second, we did not exclude studies that also included type 1 diabetes patients, but we limited the participants' ages to over 18-years-old; thus, the late depression symptoms and increased somatic burden associated with patients in the age range of T2D[25] were minimized with the exclusion of younger subjects.

Future directions

These review findings could contribute to future new approaches on the elaboration of technological strategies to cope with T2D distress and consequently improve organ complications, patient well-being and cost effectiveness in the management of T2D.

CONCLUSION

The findings of the present study show that management of T2D distress can have positive outcomes with technology-based interventions and highlight that the best results come from programs that offer not only a personalized digital/technological experience for patients but also routine caregiver support and attention.

ARTICLE HIGHLIGHTS

Research background

High diabetes distress is associated with poorer diabetes outcomes. Technological interventions have been used as modern tools of communication to improve communication and can impact diabetes self-management, engagement and adherence. Understanding the impact of programs with technological interventions regarding disease management on type 2 diabetes distress bears clinical significance.

Research motivation

Review authors disagree about the benefits of this technology in type 2 diabetes distress. We systematically reviewed randomized controlled trials that studied the impact of technology interventions on type 2 diabetes distress.

Research objectives

The goal of this study is to provide comprehensive overview of the impact of technology interventions on type 2 diabetes distress.

Research methods

We systematically searched MEDLINE, PubMed and EBSCO with the Boolean logic search terms were: (1) T2Diabetes; (2) Diabetes distress and (3) Technology OR mobile OR phone OR application OR web. We also systematically searched the reference lists of the included studies and relevant reviews.

Research results

We found nine full articles that met the inclusion criteria. Six out of nine articles showed that technology interventions had a positive impact on diabetes distress scale scores when compared with the initial data. Among these six articles, five showed a greater reduction in the diabetes distress scores from control interventions. Web-based interventions had good results when users received personalized feedback and routine caregiver support and attention.

Research conclusions

Technology-based interventions have a positive impact on type 2 diabetes distress management, and programs that include routine caregiver support and attention show the best results.

Research perspectives

These review findings could contribute to the development of new approaches on the elaboration of technological strategies to cope with type 2 diabetes distress and consequently improve treatment outcomes, resulting in patient well-being and better biological consequences in the management of type 2 diabetes.

REFERENCES

1   **Cho NH**, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, Ohlrogge AW, Malanda B. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Res Clin Pract* 2018; **138**: 271-281

2   **Fisher L**, Skaff MM, Mullan JT, Arean P, Mohr D, Masharani U, Glasgow R, Laurencin G. Clinical depression *vs* distress among patients with type 2 diabetes: not just a question of semantics. *Diabetes Care* 2007; **30**: 542-548

3   **Polonsky WH**, Fisher L, Earles J, Dudl RJ, Lees J, Mullan J, Jackson RA. Assessing psychosocial distress in diabetes: development of the diabetes distress scale. *Diabetes Care* 2005; **28**: 626-631

4   **Fisher L**, Glasgow RE, Mullan JT, Skaff MM, Polonsky WH. Development of a brief diabetes distress screening instrument. *Ann Fam Med* 2008; **6**: 246-252

5   **McGuire BE**, Morrison TG, Hermanns N, Skovlund S, Eldrup E, Gagliardino J, Kokoszka A, Matthews D, Pibernik-OkanoviÄ‡ M, Rodríguez-Saldaña J, de Wit M, Snoek FJ. Short-form measures of diabetes-related emotional distress: the Problem Areas in Diabetes Scale (PAID)-5 and PAID-1. *Diabetologia* 2010; **53**: 66-69

6   **Kubzansky LD**, Davidson KW, Rozanski A. The clinical impact of negative psychological states: expanding the spectrum of risk for coronary artery disease. *Psychosom Med* 2005; **67** Suppl 1: S10-S14

7   **Martínez-Pérez B**, de la Torre-Díez I, López-Coronado M, Herreros-González J. Mobile apps in cardiology: review. *JMIR Mhealth Uhealth* 2013; **1**: e15

8   **Maddison R**, Rawstorn JC, Shariful Islam SM, Ball K, Tighe S, Gant N, Whittaker RM, Chow CK. mHealth

Interventions for Exercise and Risk Factor Modification in Cardiovascular Disease. *Exerc Sport Sci Rev* 2019; **47**: 86-90

9   **Quinn CC**, Shardell MD, Terrin ML, Barr EA, Ballew SH, Gruber-Baldini AL. Cluster-randomized trial of a mobile phone personalized behavioral intervention for blood glucose control. *Diabetes Care* 2011; **34**: 1934-1942

10   **Or CK**, Tao D. Does the use of consumer health information technology improve outcomes in the patient self-management of diabetes? *Int J Med Inform* 2014; **83**: 320-329

11   **Hou C**, Carter B, Hewitt J, Francisa T, Mayor S. Do Mobile Phone Applications Improve Glycemic Control (HbA1c) in the Self-management of Diabetes? *Diabetes Care* 2016; **39**: 2089-2095

12   **Hunt CW**. Technology and diabetes self-management: An integrative review. *World J Diabetes* 2015; **6**: 225-233

13   **Mathiesen AS**, Thomsen T, Jensen T, Schiøtz C, Langberg H, Egerod I. The influence of diabetes distress on digital interventions for diabetes management in vulnerable people with type 2 diabetes: A qualitative study of patient perspectives. *J Clin Transl Endocrinol* 2017; **9**: 41-47

14   **Alcántara-Aragón V**. Improving patient self-care using diabetes technologies. *Ther Adv Endocrinol Metab* 2019; **10**: 2042018818824215

15   **Fisher L**, Hessler D, Masharani U, Strycker L. Impact of baseline patient characteristics on interventions to reduce diabetes distress: the role of personal conscientiousness and diabetes self-efficacy. *Diabet Med* 2014; **31**: 739-746

16   **Nobis S**, Lehr D, Ebert DD, Baumeister H, Snoek F, Riper H, Berking M. Efficacy of a web-based intervention with mobile phone support in treating depressive symptoms in adults with type 1 and type 2 diabetes: a randomized controlled trial. *Diabetes Care* 2015; **38**: 776-783

17   **Bajaj HS**, Venn K, Ye C, Aronson R. Randomized Trial of Long-Acting Insulin Glargine Titration Web Tool (LTHome) Versus Enhanced Usual Therapy of Glargine Titration (INNOVATE Trial). *Diabetes Technol Ther* 2016; **18**: 610-615

18   **Rondags SM**, de Wit M, Twisk JW, Snoek FJ. Effectiveness of HypoAware, a Brief Partly Web-Based Psychoeducational Intervention for Adults With Type 1 and Insulin-Treated Type 2 Diabetes and Problematic Hypoglycemia: A Cluster Randomized Controlled Trial. *Diabetes Care* 2016; **39**: 2190-2196

19   **Holland-Carter L**, Tuerk PW, Wadden TA, Fujioka KN, Becker LE, Miller-Kovach K, Hollander PL, Garvey WT, Weiss D, Rubino DM, Kushner RF, Malcolm RJ, Raum WJ, Hermayer KL, Veliko JL, Rost SL, Sora ND, Salyer JL, O'Neil PM. Impact on psychosocial outcomes of a nationally available weight management program tailored for individuals with type 2 diabetes: Results of a randomized controlled trial. *J Diabetes Complications* 2017; **31**: 891-897

20   **Newby J**, Robins L, Wilhelm K, Smith J, Fletcher T, Gillis I, Ma T, Finch A, Campbell L, Andrews G. Web-Based Cognitive Behavior Therapy for Depression in People With Diabetes Mellitus: A Randomized Controlled Trial. *J Med Internet Res* 2017; **19**: e157

21   **Ebert DD**, Nobis S, Lehr D, Baumeister H, Riper H, Auerbach RP, Snoek F, Cuijpers P, Berking M. The 6-month effectiveness of Internet-based guided self-help for depression in adults with Type 1 and 2 diabetes mellitus. *Diabet Med* 2017; **34**: 99-107

22   **Schlicker S**, Weisel KK, Buntrock C, Berking M, Nobis S, Lehr D, Baumeister H, Snoek FJ, Riper H, Ebert DD. Do Nonsuicidal Severely Depressed Individuals with Diabetes Profit from Internet-Based Guided Self-Help? *J Diabetes Res* 2019; **2019**: 2634094

23   **Clarke J**, Sanatkar S, Baldwin PA, Fletcher S, Gunn J, Wilhelm K, Campbell L, Zwar N, Harris M, Lapsley H, Hadzi-Pavlovic D, Christensen H, Proudfoot J. A Web-Based Cognitive Behavior Therapy Intervention to Improve Social and Occupational Functioning in Adults With Type 2 Diabetes (The SpringboarD Trial): Randomized Controlled Trial. *J Med Internet Res* 2019; **21**: e12246

24   **Clarke G**, Eubanks D, Reid E, Kelleher C, O'Connor E, DeBar LL, Lynch F, Nunley S, Gullion C. Overcoming Depression on the Internet (ODIN) (2): a randomized trial of a self-help depression skills program with reminders. *J Med Internet Res* 2005; **7**: e16

25   **Reimer A**, Schmitt A, Ehrmann D, Kulzer B, Hermanns N. Reduction of diabetes-related distress predicts improved depressive symptoms: A secondary analysis of the DIAMOS study. *PLoS One* 2017; **12**: e0181218

Prevalence and impact of diabetes in patients with COVID-19 in China

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Abstract

BACKGROUND
Coronavirus disease 2019 (COVID-19) is an emerging infectious disease that has spread rapidly around the world. Previous studies have indicated that COVID-19 patients with diabetes are prone to having poor clinical outcomes.

AIM
To systematically evaluate the prevalence of diabetes among COVID-19 patients in China and its impact on clinical outcomes, including ICU admission, progression to severe cases, or death.

METHODS
We searched studies published in PubMed, Web of Science, and EMBASE from December 1, 2019 to March 31, 2020 to identify relevant observational study that investigated the prevalence of diabetes among COVID-19 patients or its impact on clinical outcomes. We used a random-effects or fixed-effects model to estimate the pooled prevalence of diabetes and risk ratio (RR) and its 95% confidence interval (CI) of diabetes on outcomes. Funnel plots were used to evaluate the publication bias and the heterogeneity was evaluated by I^2 statistic.

RESULTS
Twenty-three eligible articles including 49564 COVID-19 patients (1573 with and 47991 without diabetes) were finally included. The pooled prevalence of diabetes was 10% (95%CI: 7%-15%) in COVID-19 patients. In the subgroup analyses, the pooled prevalence of diabetes was higher in studies with patients aged > 50 years (13%; 95%CI: 11%-16%) than in studies with patients aged ≤ 50 years (7%; 95%CI: 6%-8%), in severe patients (17%; 95%CI: 14%-20%) than in non-severe patients (6%; 95%CI: 5%-8%), and in dead patients (30%; 95%CI: 13%-46%) than in survivors (8%; 95%CI: 2%-15%) ($P < 0.05$ for all). Compared with patients without

diabetes, the risk of severe cases was higher (RR = 2.13, 95%CI: 1.76-2.56, I^2 = 49%) in COVID-19 patients with diabetes. The risk of death was also higher in COVID-19 patients with diabetes (RR = 3.16, 95%CI: 2.64-3.78, I^2 = 34%). However, diabetes was not found to be significantly associated with admission to ICU (RR = 1.16, 95%CI: 0.15-9.11).

CONCLUSION
Nearly one in ten COVID-19 patients have diabetes in China. Diabetes is associated with a higher risk of severe illness and death. The present study suggested that targeted early intervention is needed in COVID-19 patients with diabetes.

Key Words: Diabetes; COVID-19; Systematic review; Meta-analysis

Core Tip: At present, the prevalence and impact of diabetes in patients with coronavirus disease 2019 (COVID-19) have not been systematically reviewed in China. This meta-analysis for the first time focused on the pooled prevalence of diabetes among COVID-19 patients and its impact on clinical outcomes (ICU admission, severity, and death) in China. The analysis showed that the pooled prevalence of diabetes was 10% [95% confidence interval (CI): 7%-15%] in COVID-19 patients. Besides, the risks of severe cases (risk ratio = 2.13, 95%CI: 1.76-2.56, I^2 = 49%, P = 0.007) and deaths (risk ratio = 3.16, 95%CI: 2.64-3.78, I^2 = 34%, P = 0.20) were both higher in COVID-19 patients with diabetes compared with those without. Our findings highlight the need for targeted intervention on diabetes among COVID-19 patients.

INTRODUCTION

In December 2019, some pneumonia cases of unknown cause were reported in Wuhan, Hubei Province, China, and deep sequencing analysis from lower respiratory tract samples indicated a novel coronavirus that caused coronavirus disease 2019 (COVID-19)[1]. As of April 12, 2020, there were 1696588 confirmed cases of COVID-19 in the world, with 105952 deaths, according to the World Health Organization. Previous studies have reported that patients with poor immune function, such as the elderly or patients with chronic diseases, may develop severity or even death, such as diabetes and cardiovascular diseases[2,3]. Guo *et al*[4] reported that diabetes patients with COVID-19 were prone to have severe pneumonia, releasing tissue damage-related enzymes, excessively uncontrolled inflammation, and hypercoagulable states associated with abnormal glucose metabolism[4]. Similarly, COVID-19 is not conducive to blood glucose control in diabetic patients. Zhou and Tan[5] monitored the blood glucose of 26 diabetic COVID-19 patients and found that 56.6% showed abnormal blood glucose levels, 69.0% were considered to have a poor blood glucose level, and 10.3% have experienced hypoglycemia at least once[5].

As a chronic illness, diabetes is characterized by elevated levels of blood glucose, and accompanied by disturbed metabolism of fats and proteins[6]. Several studies reported the prevalence of diabetes among COVID-19 patients. In China, Shi *et al*[7] discovered that the comorbidity rate of diabetes was 5.95% in Zhengzhou, while it was 7.23% in the study by Lian *et al*[8]. Huang *et al*[9] found that the prevalence of diabetes in Wuhan was 11.8%, while Chen *et al*[10] reported the rate at 14%. Bhatraju *et al*[11] found that prevalence of diabetes among COVID-19 patients was 58% in the United States. It was reported that the prevalence of diabetes was about three-fold higher in intensive care unit (ICU) cases than in non-ICU cases[12]. The results on the prevalence of diabetes varied across different studies.

Although epidemiological data on COVID-19 infection are growing, the prevalence

and impact of diabetes in COVID-19 patients have not been systematically reviewed at the country level. Thus, we conducted a systematic review and meta-analysis to assess the prevalence of diabetes among COVID-19 patients in China and identify its impact on clinical outcomes.

MATERIALS AND METHODS

According to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement, we performed this systematic review and meta-analysis[13].

Data sources and search strategy

The literature was searched independently by two investigators (Du M and Yan WX), which was published online from December 1, 2019 to March 31, 2020, from three databases including PubMed, Web of Science, and EMBASE using the following search term: ("novel coronavirus", "2019-nCoV", "coronavirus disease 2019", "COVID-19", or "SARS-CoV-2") and "diabetes". There was no limitation to language or region. Moreover, we also searched highly relevant reference articles by reviewing the list of references. To exclude duplicates, EndNote X 9.0 software was used to manage the records.

Inclusion and exclusion criteria

The inclusion criteria for articles in the meta-analysis included: (1) Cross-sectional studies or cohort studies; and (2) Studies that reported the prevalence of diabetes in COVID-19 patients. The exclusion criteria were as follows: (1) The subjects of the study were irrelevant (animal experiments, pathological researches, or molecular researches); (2) Reviews, editorials, or case reports; (3) Overlapped studies. The latest one was selected if studies overlapped; (4) Studies that were not conducted in China; (5) Duplicated studies; and (6) Key information could not be extracted, for example the prevalence of diabetes.

Two investigators (Lin YX and Yan WX) independently identified the studies, based on the inclusion and exclusion criteria. When discrepancies occurred, they were solved by consensus or decided by a third investigators (DM).

Data extraction and study quality assessment

The primary outcomes were prevalence of diabetes among COVID-19 patients and its impact on clinical outcomes (admission to ICU, severe cases, and death). In view of the piloted forms, the information which was extracted independently by two investigators (Du M and Yan WX) from the selected studies included three main parts: (1) Basic information of the studies comprising first author, publication year, survey time, study design, and journal; (2) Characteristics of the study population including sample size, location, median/mean age, gender ratio; and (3) Primary outcomes including the numbers of patients with diabetes and non-diabetes in the total COVID-19 patients and in different subgroups (ICU patients *vs* non-ICU patients, severe *vs* non-severe patients, and dead patients *vs* survivors). The methodological quality of the included studies was evaluated by using the tool developed by Hoy and colleagues[14], which had been used in other meta-analyses for pooled prevalence[15]. To generate an overall quality score that ranged from 0 to 10, we assigned each item a score of 1 (yes) or 0 (no), and summed scores across items. Then, according to the overall scores, the methodological quality of studies was classified into three levels: Low (> 8), moderate (6–8), or high (≤ 5) risk of bias[14]. The study quality was independently assessed by two investigators (Du M and Lin YX), and disagreements were resolved by consensus.

Data synthesis and statistical analysis

In order to get an overall summary estimate of diabetes prevalence across studies, a meta-analysis was used to summarize prevalence data and a random-effects or fixed-effects model was used to pool the study-specific estimates. We used the I^2 statistic to assess the magnitude of heterogeneity, with 25%, 50%, and 75% heterogeneity representing low, moderate, and high degrees of heterogeneity, respectively[16]. According to the analysis results, the proper effect model was selected: If $I^2 \leq 50\%$, the fixed-effects model was used, otherwise the random-effects model was used.

If substantial heterogeneity was detected, to investigate the possible sources of heterogeneity, subgroup analysis was performed by using the following grouping variables: Sample size, location, age, admission to ICU or not, severe cases or not, and death or not. Subgroup comparisons were made using the Q test. A *P* value less than

0.05 was considered to indicate a significant difference between subgroups. We performed sensitivity analysis by deleting the lowest quality score of study and by using a different model (fixed-effect or random-effect model). The risk ratio (RR) and the corresponding 95% confidence interval (CI) were calculated to quantify the impact of diabetes on clinical outcomes (ICU *vs* non-ICU patients, severe *vs* non-severe patients, and dead patients *vs* survivors), and a *P* value < 0.05 was deemed significant. We used forest plots to describe the pooled rate of diabetes and the risk ratio of diabetes to related outcomes. To assess publication bias, we used funnel plots and Egger' publication bias test. We analyzed data using R version 3.5.3 and Stata version 16.0.

RESULTS

Study selection and study characteristics
One hundred and ninety articles were searched totally, of which 64 reported duplicate results. After skimming the title and abstracts, we excluded 101 reviews, guidelines, or irrelevant studies. After reading the full articles, six articles that provided insufficient information were excluded. Four articles from other sources were included. As a result, 23 eligible articles were included in the meta-analysis[1,3,4,7-9,17-34]. Figure 1 shows the flow chart of study selection.

The total sample size of the included studies was 49564, with the number of subjects in each study varying from 18 to 44672. Baseline characteristics are shown in Table 1.

Pooled prevalence of diabetes and subgroup analysis
Among the 23 studies included, the rate of diabetes varied widely from 2.47% to 22.22%. The pooled prevalence of diabetes was 10% (7%-15%) among 49564 COVID-19 patients from 23 studies (Figure 2). For the subgroup analyses, the pooled prevalence of diabetes was higher in studies on patients with a median age > 50 years (13%; 95%CI: 11%-16%) than in those on patients with a median age ≤ 50 years (7%; 95%CI: 6%-8%), in severe patients (17%; 95%CI: 14%-20%) than in non-severe patients (6%; 95%CI: 5%-8%), and in dead patients (30%; 95%CI: 13%-46%) than in survivors (8%; 95%CI: 2%-15%, Table 2).

Impact of diabetes on clinical outcomes
Compared with patients without diabetes, the risks of severe cases (RR = 2.13, 95%CI: 1.76-2.56; I^2 = 49%) and deaths (RR = 3.16, 95%CI: 2.64-3.78, I^2 = 34%) were both higher in COVID-19 patients with diabetes (Figure 3B and C). However, there was no relationship between diabetes and admission to ICU (RR = 1.16, 95%CI: 0.15-9.11; Figure 3A).

In the sensitivity analysis, we found the pooled results of meta-analysis had no difference between the fixed-effects model and random-effects model or when deleting the study with the lowest quality score, which showed that the results of pooled diabetes prevalence and RRs were stable. Both funnel plots (Figure 4B and C) and Egger's tests showed publication bias on the pooled prevalence of diabetes (*P* < 0.001; Figure 5) and the association of diabetes and ICU admission (*P* < 0.05; Figure 4A), while showed no evidence of publication bias on the association of diabetes and severe cases (*t* = -1.36, *P* = 0.23) or death (*t* = 0.03, *P* = 0.98).

DISCUSSION

Diabetes is an important public health problem. Uncontrolled diabetes could lead to complications in many organs, resulting in loss of vision and kidney function, heart attacks, strokes, and lower limb amputations which cause disability and death[6]. Previous studies reported that the presence of comorbidities in severe acute respiratory syndrome patients increased the risk of death by nearly two-fold[35]. Yuan *et al*[31] found that the incidence of comorbidities in the death group was significantly higher than that of the survival group (80% *vs* 29%, *P* = 0.018), especially for comorbid diabetes, hypertension, and heart disease[31]. Some studies suggested that chronic diseases such as hypertension and diabetes might be important risk factors for poor prognosis in patients with COVID-19[36]. In this systematic review and meta-analysis, we included 23 studies related to COVID-19 cases and diabetes. Our results showed that the prevalence of diabetes in COVID-19 cases was 10%. The pooled prevalence of

Table 1 Baseline characteristics of the 23 included studies

Ref.	Survey period	Sample size	Location	Age (mean or median)	Sex (male, %)	Prevalence of diabetes (%)	Quality score
Chen *et al*[17]	2020.1.1-2020.1.20	99	Wuhan China	55.5	67.68	12.12	7
Huang *et al*[1]	2019.12.16-2010.1.2	41	Wuhan China	49.0	73.17	19.51	7
Liu *et al*[18]	2019.12.30-2020.1.24	137	Wuhan China	57	44.53	10.22	7
Wang *et al*[19]	2020.1-2020.1.31	138	Wuhan China	56	54.35	10.14	7
Zhang *et al*[20]	2020.1.06-2020.2.3	140	Wuhan China	57	50.71	12.14	7
Chen *et al*[21]	2019.1 -2020 .2	150	Wuhan China	59	56.00	13.33	5
Guan *et al*[22]	2020.12.11-1.31	1590	China	48.9	56.86	8.18	9
Guo *et al*[23]	2020.1.23-2020.2.23	187	Wuhan China	58.5	48.66	14.97	7
Guo *et al*[4]	2020.2.10-2020.2.29	174	Wuhan China	59	43.68	21.26	6
Huang *et al*[9]	2019.12.21-2020.1.28	34	Wuhan China	56.24	41.18	11.76	4
China CDC[24]	As of 2020.2.11	44672	China	-	51.44	2.47	6
Lian *et al*[8]	2020.1.17-2020.2.12	788	Zhejiang China	45.8	51.65	7.23	7
Shi *et al*[7]	As of 2020.2.17	487	Zhejiang China	46	53.18	5.95	3
Li *et al*[25]	2020.1.18-2020.2.7	78	Zhuhai China	44.6	48.72	5.13	5
Liu *et al*[3]	2020.1.15-2020.2.18	56	Hainan China	53.75	55.36	7.14	6
Wan *et al*[26]	2020.1.23-2020.2.28	135	Chongqing China	47	53.33	8.89	5
Wang *et al*[27]	2020.1.21-2020.2.5	18	Zhengzhou China	39	55.56	16.67	5
Wu *et al*[28]	2019.12.25-2020.1.26	201	Wuhan China	51	63.68	10.95	7
Xu *et al*[29]	2020.1.23-2020.2.4	90	Guangzhou China	50	43.33	5.56	6
Yuan *et al*[30]	2020.1.11-2020.2.4	94	Shenzhen China	40	44.68	5.32	6
Yuan *et al*[31]	2020.1.1-2020.1.25	27	Wuhan China	60	44.44	22.22	5
Zhao *et al*[32]	2020.1.23-2020.1.31	37	Wuhan China	41	37.84	10.81	6
Zhou *et al*[33]	2019.12.29-2020.1.31	191	Wuhan China	56	62.30	18.85	7

One study[34] only reporting information on severe patients is not shown in the table. CDC: Centers for disease control.

diabetes in COVID-19 cases in our study (10%) was slightly higher than the estimation in the general population (8.5%)[6]. Li *et al*[12] found that the prevalence of diabetes is about 9.7% in COVID-19 cases by meta-analysis including five studies[12], which was similar to our findings. Yang *et al*[37] found that the prevalence of diabetes is about 9% by a meta-analysis that include eight studies. The differences between these studies might be related to the number of studies included, sample size, and characteristics of the included patients.

We found that diabetes was associated with severe illness (RR = 2.13, 95%CI: 1.76, 2.56) and death (RR = 3.16, 95%CI: 2.64-3.78). A meta-analysis by Li *et al*[12] reported that the prevalence of diabetes in ICU patients was not significantly higher than that in non-ICU patients (RR = 2.21, 95%CI: 0.88, 5.57)[12]. Yang *et al*[37] found that the

Table 2 Pooled prevalence of diabetes among coronavirus disease 2019 patients in China

Item	Number of studies	Sample size	Diabetes	Prevalence(95%CI, %)	I^2	P values	
						Heterogeneity	Subgroup difference
Overall	23	49564	1573	10 (7-15)	97%	< 0.01	-
Subgroup analysis							
Sample size							0.002
≤ 100	10	574	55	9 (6-11)	28%	0.14	
100-200	8	1252	178	14 (11-16)	60%	0.02	
> 200	5	49588	1340	7 (4-9)	94%	< 0.0001	
Location							< 0.0001
Wuhan, China	13	1556	222	14 (12-16)	35%	0.12	
Outside of Wuhan, China	8	1746	119	7 (6-8)	0%	0.79	
China (all cities)	2	46262	1432	5 (0-11)	99%	< 0.0001	
Age group							< 0.0001
≤ 50 yr	10	3358	257	7 (6-8)	10%	0.27	
> 50 yr	12	1534	214	13 (11-16)	45%	0.05	
ICU admission							0.94
ICU	2	112	20	15 (4-26)	47%	0.17	
Non-ICU	2	1519	118	14 (3-35)	78%	0.03	
Severity							< 0.0001
Severe	8	553	99	17 (14-20)	0%	0.92	
Non-severe	7	2190	143	6 (5-8)	41%	0.05	
Death							0.02
Death	5	1146	120	30 (13-46)	89%	< 0.0001	
Survival	5	45508	1191	8 (2-15)	99%	< 0.0001	

ICU: Intensive care unit.

proportion of diabetic patients between severe and non-severe patients had no significant difference by meta-analysis[37]. These two meta-analyses described the prevalence of diabetes among COVID-19 patients with limited studies (less than 10 studies) included in the analysis. Huang *et al*[38] reported that diabetes was associated with severe illness (RR = 2.45, 95%CI: 1.79-3.35) in a meta-analysis of 30 studies, which was similar to our findings[38]. It should be noted that, the pooled prevalence of diabetes was 30% (95%CI: 12%-51%) in the dead patients, which was much higher than 8% (95%CI: 3%-14%) in the survivors in our study. Pooled RR for the association between diabetes and death was 3.16 (95%CI: 2.64-3.78), which was similar to the study of Kumar *et al*[39], who did a meta-analysis of 33 case-control studies to examine the association between diabetes and mortality in COVID-19 patients. They found that diabetes was significantly associated with mortality of COVID-19 (RR = 1.90, 95%CI: 1.37-2.64)[39]. The findings indicated that the risk of death among COVID-19 patients with diabetes should be paid more attention.

Diabetes and susceptibility of the body have certain physiological mechanisms. Because of the accumulation of activated innate immune cells in metabolic tissues, the inflammatory mediators such as IL-1β and TNF-α are released, which accelerates β-cell damage and systemic insulin resistance. Conversely, metabolic problems may further impair the immunologic function of macrophages and lymphocytes, which makes the individual easy to develop other complications[40]. Recently, it was found that the proportion of CD3+ or CD4+ T cells and the absolute count of CD3+, CD4+, or CD8+ T cells in the death group were significantly higher than those in the survival group by analyzing the clinical data of viral pneumonia patients retrospectively, and the death group had higher levels of different inflammatory factors compared to the survival
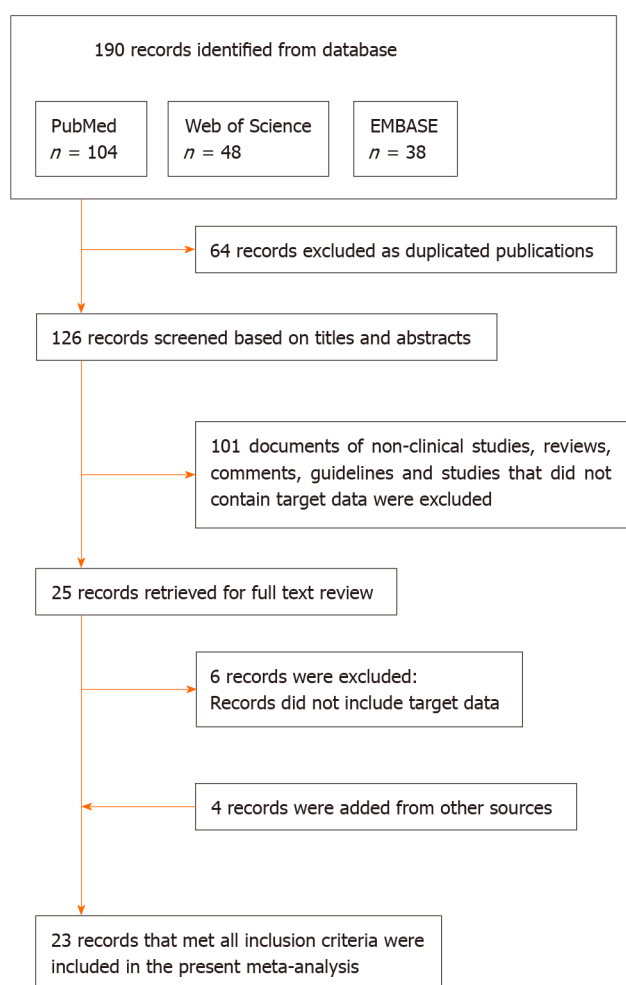
Figure 1 Flow diagram of the study selection process.

group[17]. Huang *et al*[1] also found that the plasma concentrations of inflammatory factors (such as IL-2, IL-7, and IL-10) in ICU patients with COID-19 were higher than those of the non-ICU COVID-19 patients[1]. Studies have found that coronaviruses (including SARS-CoV and SARS-CoV-2) can bind to target cells because of angiotensin-converting enzyme 2 (ACE2), which may promote the proliferation of SARS-CoV-2and enhance its ability to infect[41]. ACE inhibitors and type I angiotensin II receptor blockers (ARBs) that are used in patients with type 1 or type 2 diabetes can result in a significant increase in ACE2[41,42]. Besides ACE2, dipeptidyl peptidase-4 (DPP4) was also found to be a coronavirus receptor protein[43]. DPP4 inhibitors, one of the glucose-lowering agents, are widely used in treatment of diabetes and known to modify the biological activities of multiple immunomodulatory substrates[44]. The potential role of DPP4 inhibition in preventing SARS-CoV-2 infection and progression needs to be clarified in the future[44]. Previous studies found that ACE2 and DPP4 were established transducers of metabolic signals and pathways regulating inflammation and glucose homeostasis[43]. Guo *et al*[45] analyzed the biochemical indicators of diabetic and non-diabetic patients in new coronavirus cases and found that compared with non-diabetic patients, the levels of serum inflammation-related biomarkers, including IL-6, C-reactive protein, serum ferritin, and D-dimer increased significantly in diabetic patients ($P < 0.01$), suggesting that patients with diabetes might have a worse prognosis[45]. Diabetes may not be related to the risk of COVID-19 infection, but it could deteriorate clinical outcome of COVID-19 patients[46].

This study has some limitations. First, publication bias might exit in this meta-analysis. Second, due to the limitation of sample size and short study periods, limited studies had completed follow-up of the COVID-19 patients and reported the results of its impact on clinical outcomes.
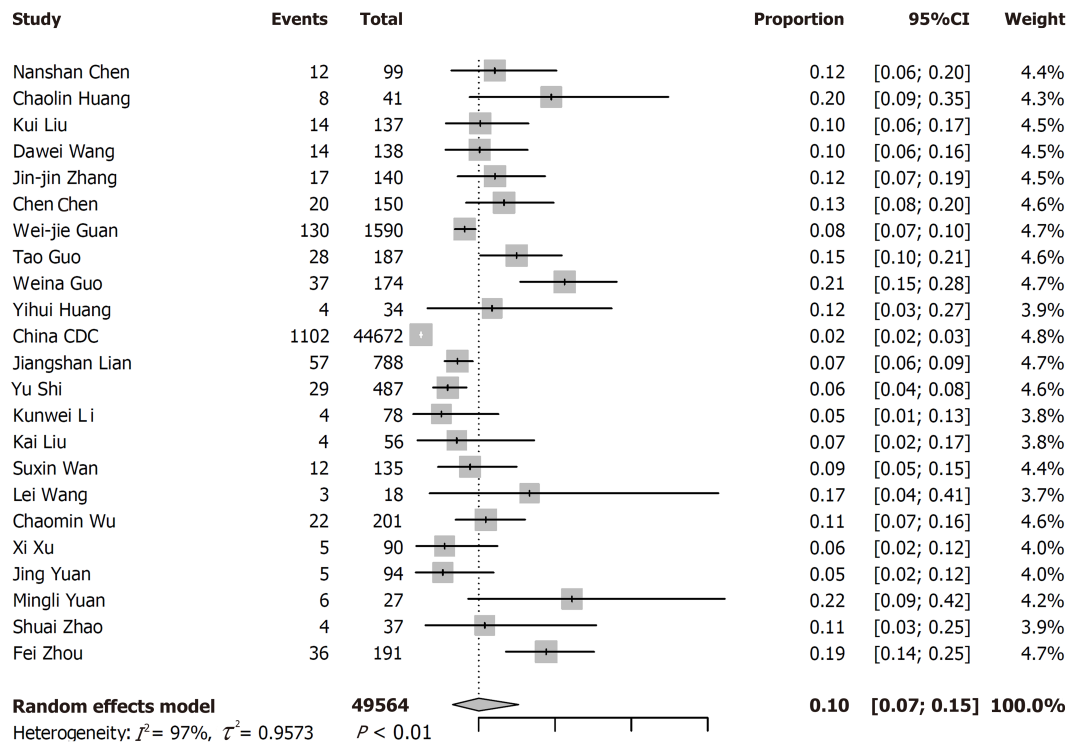
Study	Events	Total	Proportion	95%CI	Weight
Nanshan Chen	12	99	0.12	[0.06; 0.20]	4.4%
Chaolin Huang	8	41	0.20	[0.09; 0.35]	4.3%
Kui Liu	14	137	0.10	[0.06; 0.17]	4.5%
Dawei Wang	14	138	0.10	[0.06; 0.16]	4.5%
Jin-jin Zhang	17	140	0.12	[0.07; 0.19]	4.5%
Chen Chen	20	150	0.13	[0.08; 0.20]	4.6%
Wei-jie Guan	130	1590	0.08	[0.07; 0.10]	4.7%
Tao Guo	28	187	0.15	[0.10; 0.21]	4.6%
Weina Guo	37	174	0.21	[0.15; 0.28]	4.7%
Yihui Huang	4	34	0.12	[0.03; 0.27]	3.9%
China CDC	1102	44672	0.02	[0.02; 0.03]	4.8%
Jiangshan Lian	57	788	0.07	[0.06; 0.09]	4.7%
Yu Shi	29	487	0.06	[0.04; 0.08]	4.6%
Kunwei Li	4	78	0.05	[0.01; 0.13]	3.8%
Kai Liu	4	56	0.07	[0.02; 0.17]	3.8%
Suxin Wan	12	135	0.09	[0.05; 0.15]	4.4%
Lei Wang	3	18	0.17	[0.04; 0.41]	3.7%
Chaomin Wu	22	201	0.11	[0.07; 0.16]	4.6%
Xi Xu	5	90	0.06	[0.02; 0.12]	4.0%
Jing Yuan	5	94	0.05	[0.02; 0.12]	4.0%
Mingli Yuan	6	27	0.22	[0.09; 0.42]	4.2%
Shuai Zhao	4	37	0.11	[0.03; 0.25]	3.9%
Fei Zhou	36	191	0.19	[0.14; 0.25]	4.7%
Random effects model	**49564**		**0.10**	**[0.07; 0.15]**	**100.0%**

Heterogeneity: $I^2 = 97\%$, $\tau^2 = 0.9573$ $P < 0.01$

Figure 2 Meta-analysis for the prevalence of diabetes in coronavirus disease 2019 cases in China.

CONCLUSION

This study indicated that nearly one in ten COVID-19 patients have diabetes and diabetes increases the risk of severe illness and death. Targeted public health measures should be carried out timely to make the protection of patients with diabetes from COVID-19 and other respiratory infections even better. More adequate and vigorous research should be conducted to prove the associations found in this study.
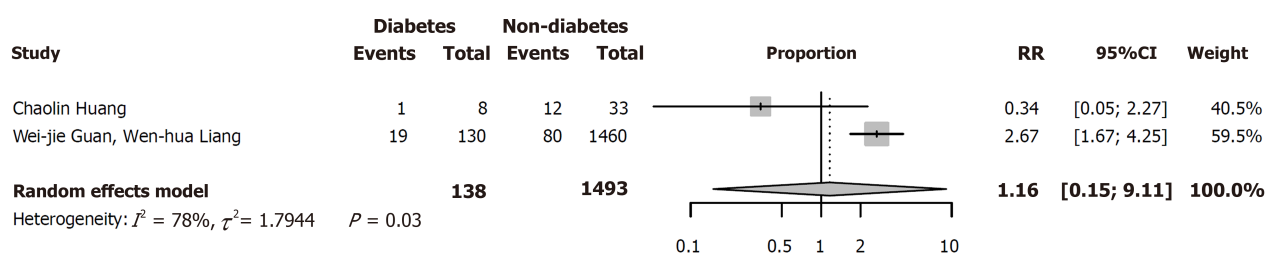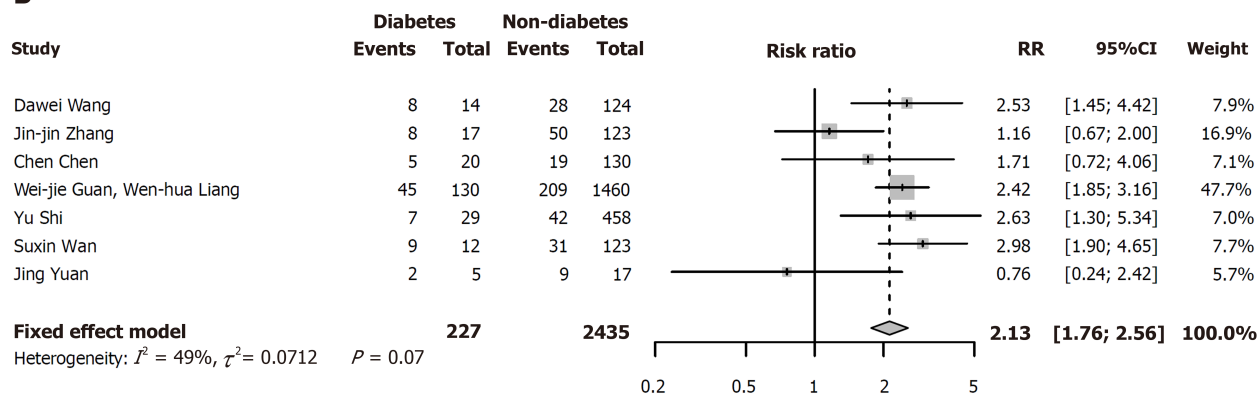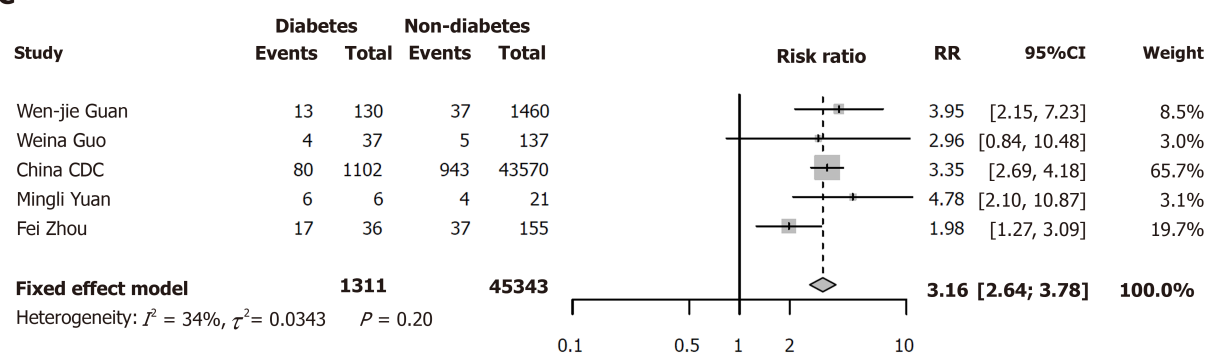
A

B

C

Figure 3 Forest plots for impact of diabetes on clinical outcomes. A: Intensive care unit *vs* non-intensive care unit; B: Severe *vs* non-severe; C: Death *vs* survival. RR: Risk ratio; CI: Confidence interval.
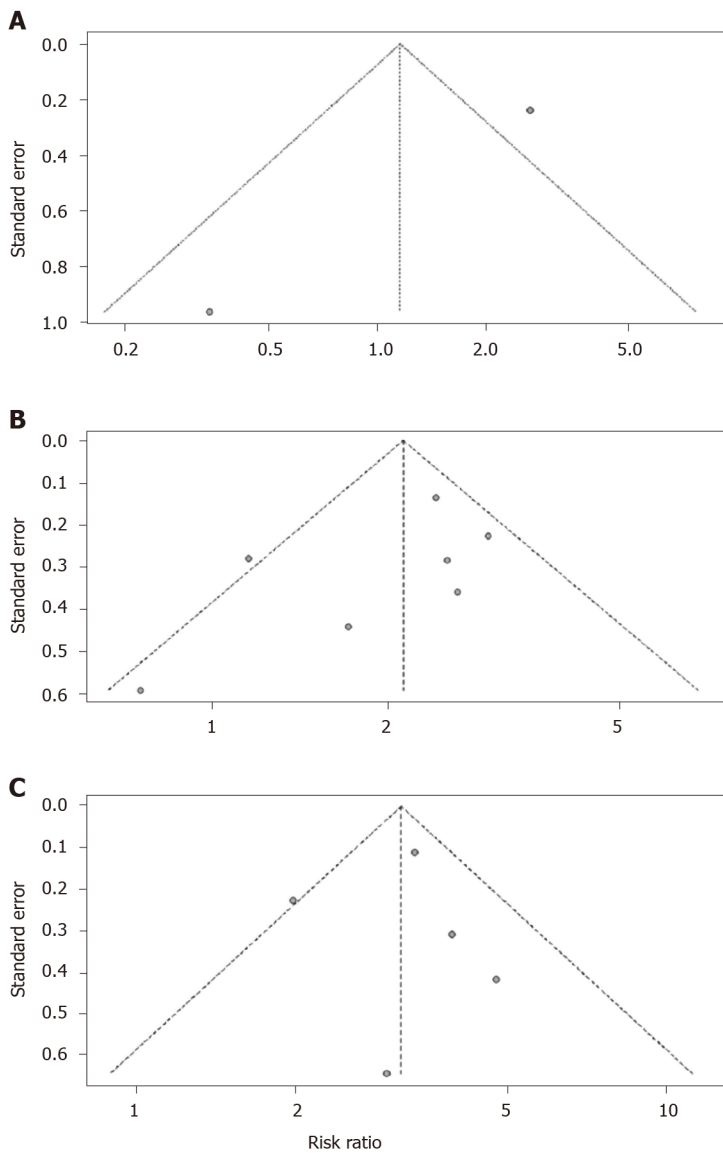
Figure 4 Funnel plots for impact of diabetes on clinical outcomes. A: Intensive care unit *vs* non-intensive care unit; B: Severe *vs* non-severe; and C: Death *vs* survival.
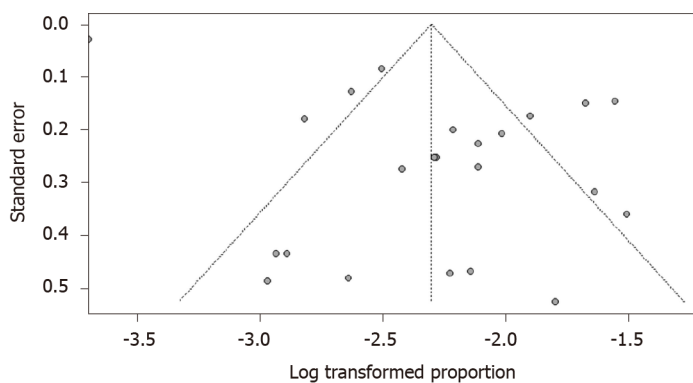

Figure 5 Funnel plot for the prevalence of diabetes in coronavirus disease 2019 cases.

ARTICLE HIGHLIGHTS

Research background

Coronavirus disease 2019 (COVID-19) has spread around the world rapidly. The prevalence of diabetes varies across different studies. Previous studies showed that COVID-19 patients with diabetes were prone to having poor clinical outcomes. However, a systematical review of the prevalence of diabetes in COVID-19 patients and the impact of diabetes on clinical outcomes has not been done in China.

Research motivation

We hypothesized that the presence of diabetes is associated with a poor prognosis. To our knowledge, this is the first meta-analysis which focused on evaluating the prevalence of diabetes among patients with COVID-19 infection in China and its impact on clinical outcomes.

Research objectives

The aim of this study was to systematically evaluate the prevalence of diabetes among COVID-19 patients in China and its impact on clinical outcomes, including ICU admission, progression to severe cases, or death.

Research methods

A systematic review and meta-analysis of observational studies were performed.

Research results

Twenty-three eligible articles including 49564 COVID-19 patients (1573 with and 47991 without diabetes) were included. The pooled prevalence of diabetes was 10% [95% confidence interval (CI): 7%-15%] in COVID-19 patients. In the subgroup analyses, the pooled prevalence of diabetes was higher in studies on patients with a median age > 50 years (13%; 95%CI: 11%-16%) than studies on patients with a median age ≤ 50 years (7%; 95%CI: 6%-8%), in severe patients (17%; 95%CI: 14%-20%) than in non-severe patients (6%; 95%CI: 5%-8%), and in dead patients (30%; 95%CI: 13%-46%) than in survivors (8%; 95%CI: 2%-15%, all $P < 0.05$). Compared with patients without diabetes, the risks of severe cases [risk ratio (RR) = 2.13, 95%CI: 1.76-2.56, I^2 = 49%] and death (RR = 3.16, 95%CI: 2.64-3.78, I^2 = 34%) were both higher in COVID-19 patients with diabetes. However, diabetes was not found to be significantly associated with admission to ICU (RR = 1.16, 95%CI: 0.15-9.11).

Research conclusions

Nearly one in ten COVID-19 patients have diabetes in China. Diabetes is associated with a higher risk of severe illness and death. The present study suggested that targeted early intervention is needed in COVID-19 patients with diabetes.

Research perspectives

A systematic review and meta-analysis of observational studies which summarizes the evidence on this topic is meaningful. More adequate and vigorous research should be conducted to prove the associations found in this study.

REFERENCES

1   **Huang C**, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, Cheng Z, Yu T, Xia J, Wei Y, Wu W, Xie X, Yin W, Li H, Liu M, Xiao Y, Gao H, Guo L, Xie J, Wang G, Jiang R, Gao Z, Jin Q, Wang J, Cao B. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020; **395**: 497-506 [PMID: 31986264 DOI: 10.1016/S0140-6736(20)30183-5]
2   **Epidemiology Working Group for NCIP Epidemic Response**, Chinese Center for Disease Control and Prevention. [The epidemiological characteristics of an outbreak of 2019 novel coronavirus diseases (COVID-19) in China]. *Zhonghua Liu Xing Bing Xue Za Zhi* 2020; **41**: 145-151 [PMID: 32064853 DOI: 10.3760/cma.j.issn.0254-6450.2020.02.003]
3   **Liu K**, Chen Y, Lin R, Han K. Clinical features of COVID-19 in elderly patients: A comparison with young and middle-aged patients. *J Infect* 2020; **80**: e14-e18 [PMID: 32171866 DOI: 10.1016/j.jinf.2020.03.005]
4   **Guo W**, Li M, Dong Y, Zhou H, Zhang Z, Tian C, Qin R, Wang H, Shen Y, Du K, Zhao L, Fan H, Luo S, Hu D. Diabetes is a risk factor for the progression and prognosis of COVID-19. *Diabetes Metab Res Rev* 2020; e3319 [PMID: 32233013 DOI: 10.1002/dmrr.3319]
5   **Zhou J**, Tan J. Diabetes patients with COVID-19 need better blood glucose management in Wuhan, China. *Metabolism* 2020; **107**: 154216 [PMID: 32220612 DOI: 10.1016/j.metabol.2020.154216]

6   **World Health Organization**. Global report on diabetes 2016. Available from: https://www.who.int/diabetes/global-report/en/

7   **Shi Y**, Yu X, Zhao H, Wang H, Zhao R, Sheng J. Host susceptibility to severe COVID-19 and establishment of a host risk score: findings of 487 cases outside Wuhan. *Crit Care* 2020; **24**: 108 [PMID: 32188484 DOI: 10.1186/s13054-020-2833-7]

8   **Lian J**, Jin X, Hao S, Cai H, Zhang S, Zheng L, Jia H, Hu J, Gao J, Zhang Y, Zhang X, Yu G, Wang X, Gu J, Ye C, Jin C, Lu Y, Yu X, Yu X, Ren Y, Qiu Y, Li L, Sheng J, Yang Y. Analysis of Epidemiological and Clinical Features in Older Patients With Coronavirus Disease 2019 (COVID-19) Outside Wuhan. *Clin Infect Dis* 2020; **71**: 740-747 [PMID: 32211844 DOI: 10.1093/cid/ciaa242]

9   **Huang Y**, Tu M, Wang S, Chen S, Zhou W, Chen D, Zhou L, Wang M, Zhao Y, Zeng W, Huang Q, Xu H, Liu Z, Guo L. Clinical characteristics of laboratory confirmed positive cases of SARS-CoV-2 infection in Wuhan, China: A retrospective single center analysis. *Travel Med Infect Dis* 2020; 101606 [PMID: 32114074 DOI: 10.1016/j.tmaid.2020.101606]

10   **Chen R**, Zhang Y, Huang L, Cheng BH, Xia ZY, Meng QT. Safety and efficacy of different anesthetic regimens for parturients with COVID-19 undergoing Cesarean delivery: a case series of 17 patients. *Can J Anaesth* 2020; **67**: 655-663 [PMID: 32180175 DOI: 10.1007/s12630-020-01630-7]

11   **Bhatraju PK**, Ghassemieh BJ, Nichols M, Kim R, Jerome KR, Nalla AK, Greninger AL, Pipavath S, Wurfel MM, Evans L, Kritek PA, West TE, Luks A, Gerbino A, Dale CR, Goldman JD, O'Mahony S, Mikacenic C. Covid-19 in Critically Ill Patients in the Seattle Region - Case Series. *N Engl J Med* 2020; **382**: 2012-2022 [PMID: 32227758 DOI: 10.1056/NEJMoa2004500]

12   **Li B**, Yang J, Zhao F, Zhi L, Wang X, Liu L, Bi Z, Zhao Y. Prevalence and impact of cardiovascular metabolic diseases on COVID-19 in China. *Clin Res Cardiol* 2020; **109**: 531-538 [PMID: 32161990 DOI: 10.1007/s00392-020-01626-9]

13   **Moher D**, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med* 2009; **6**: e1000097 [PMID: 19621072 DOI: 10.1371/journal.pmed.1000097]

14   **Hoy D**, Brooks P, Woolf A, Blyth F, March L, Bain C, Baker P, Smith E, Buchbinder R. Assessing risk of bias in prevalence studies: modification of an existing tool and evidence of interrater agreement. *J Clin Epidemiol* 2012; **65**: 934-939 [PMID: 22742910 DOI: 10.1016/j.jclinepi.2011.11.014]

15   **Noubiap JJ**, Essouma M, Bigna JJ, Jingi AM, Aminde LN, Nansseu JR. Prevalence of elevated blood pressure in children and adolescents in Africa: a systematic review and meta-analysis. *Lancet Public Health* 2017; **2**: e375-e386 [PMID: 29253478 DOI: 10.1016/S2468-2667(17)30123-8]

16   **Higgins JP**, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med* 2002; **21**: 1539-1558 [PMID: 12111919 DOI: 10.1002/sim.1186]

17   **Chen N**, Zhou M, Dong X, Qu J, Gong F, Han Y, Qiu Y, Wang J, Liu Y, Wei Y, Xia J, Yu T, Zhang X, Zhang L. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* 2020; **395**: 507-513 [PMID: 32007143 DOI: 10.1016/S0140-6736(20)30211-7]

18   **Liu K**, Fang YY, Deng Y, Liu W, Wang MF, Ma JP, Xiao W, Wang YN, Zhong MH, Li CH, Li GC, Liu HG. Clinical characteristics of novel coronavirus cases in tertiary hospitals in Hubei Province. *Chin Med J (Engl)* 2020; **133**: 1025-1031 [PMID: 32044814 DOI: 10.1097/CM9.0000000000000744]

19   **Wang D**, Hu B, Hu C, Zhu F, Liu X, Zhang J, Wang B, Xiang H, Cheng Z, Xiong Y, Zhao Y, Li Y, Wang X, Peng Z. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. *JAMA* 2020 [PMID: 32031570 DOI: 10.1001/jama.2020.1585]

20   **Zhang JJ**, Dong X, Cao YY, Yuan YD, Yang YB, Yan YQ, Akdis CA, Gao YD. Clinical characteristics of 140 patients infected with SARS-CoV-2 in Wuhan, China. *Allergy* 2020; **75**: 1730-1741 [PMID: 32077115 DOI: 10.1111/all.14238]

21   **Chen C**, Chen C, Yan JT, Zhou N, Zhao JP, Wang DW. [Analysis of myocardial injury in patients with COVID-19 and association between concomitant cardiovascular diseases and severity of COVID-19]. *Zhonghua Xin Xue Guan Bing Za Zhi* 2020; **48**: E008 [PMID: 32141280 DOI: 10.3760/cma.j.cn112148-20200225-00123]

22   **Guan WJ**, Liang WH, Zhao Y, Liang HR, Chen ZS, Li YM, Liu XQ, Chen RC, Tang CL, Wang T, Ou CQ, Li L, Chen PY, Sang L, Wang W, Li JF, Li CC, Ou LM, Cheng B, Xiong S, Ni ZY, Xiang J, Hu Y, Liu L, Shan H, Lei CL, Peng YX, Wei L, Liu Y, Hu YH, Peng P, Wang JM, Liu JY, Chen Z, Li G, Zheng ZJ, Qiu SQ, Luo J, Ye CJ, Zhu SY, Cheng LL, Ye F, Li SY, Zheng JP, Zhang NF, Zhong NS, He JX, China Medical Treatment Expert Group for COVID-19. Comorbidity and its impact on 1590 patients with COVID-19 in China: a nationwide analysis. *Eur Respir J* 2020; **55** [PMID: 32217650 DOI: 10.1183/13993003.00547-2020]

23   **Guo T**, Fan Y, Chen M, Wu X, Zhang L, He T, Wang H, Wan J, Wang X, Lu Z. Cardiovascular Implications of Fatal Outcomes of Patients With Coronavirus Disease 2019 (COVID-19). *JAMA Cardiol* 2020 [PMID: 32219356 DOI: 10.1001/jamacardio.2020.1017]

24   **Meo SA**, Alhowikan AM, Al-Khlaiwi T, Meo IM, Halepoto DM, Iqbal M, Usmani AM, Hajjar W, Ahmed N. Novel coronavirus 2019-nCoV: prevalence, biological and clinical characteristics comparison with SARS-CoV and MERS-CoV. *Eur Rev Med Pharmacol Sci* 2020; **24**: 2012-2019 [PMID: 32141570 DOI: 10.26355/eurrev_202002_20379]

25   **Li K**, Fang Y, Li W, Pan C, Qin P, Zhong Y, Liu X, Huang M, Liao Y, Li S. CT image visual quantitative evaluation and clinical classification of coronavirus disease (COVID-19). *Eur Radiol* 2020; **30**: 4407-4416 [PMID: 32215691 DOI: 10.1007/s00330-020-06817-6]

26   **Wan S**, Xiang Y, Fang W, Zheng Y, Li B, Hu Y, Lang C, Huang D, Sun Q, Xiong Y, Huang X, Lv J, Luo Y, Shen L, Yang H, Huang G, Yang R. Clinical features and treatment of COVID-19 patients in northeast Chongqing. *J Med Virol* 2020; **92**: 797-806 [PMID: 32198776 DOI: 10.1002/jmv.25783]

27   **Wang L**, Gao YH, Lou LL, Zhang GJ. The clinical dynamics of 18 cases of COVID-19 outside of Wuhan, China. *Eur Respir J* 2020; **55** [PMID: 32139464 DOI: 10.1183/13993003.00398-2020]

28   **Wu C**, Chen X, Cai Y, Xia J, Zhou X, Xu S, Huang H, Zhang L, Zhou X, Du C, Zhang Y, Song J, Wang S,

Chao Y, Yang Z, Xu J, Zhou X, Chen D, Xiong W, Xu L, Zhou F, Jiang J, Bai C, Zheng J, Song Y. Risk Factors Associated With Acute Respiratory Distress Syndrome and Death in Patients With Coronavirus Disease 2019 Pneumonia in Wuhan, China. *JAMA Intern Med* 2020 [PMID: 32167524 DOI: 10.1001/jamainternmed.2020.0994]

29 **Xu X**, Yu C, Qu J, Zhang L, Jiang S, Huang D, Chen B, Zhang Z, Guan W, Ling Z, Jiang R, Hu T, Ding Y, Lin L, Gan Q, Luo L, Tang X, Liu J. Imaging and clinical features of patients with 2019 novel coronavirus SARS-CoV-2. *Eur J Nucl Med Mol Imaging* 2020; **47**: 1275-1280 [PMID: 32107577 DOI: 10.1007/s00259-020-04735-9]

30 **Yuan J**, Zou R, Zeng L, Kou S, Lan J, Li X, Liang Y, Ding X, Tan G, Tang S, Liu L, Liu Y, Pan Y, Wang Z. The correlation between viral clearance and biochemical outcomes of 94 COVID-19 infected discharged patients. *Inflamm Res* 2020; **69**: 599-606 [PMID: 32227274 DOI: 10.1007/s00011-020-01342-0]

31 **Yuan M**, Yin W, Tao Z, Tan W, Hu Y. Association of radiologic findings with mortality of patients infected with 2019 novel coronavirus in Wuhan, China. *PLoS One* 2020; **15**: e0230548 [PMID: 32191764 DOI: 10.1371/journal.pone.0230548]

32 **Zhao S**, Ling K, Yan H, Zhong L, Peng X, Yao S, Huang J, Chen X. Anesthetic Management of Patients with COVID 19 Infections during Emergency Procedures. *J Cardiothorac Vasc Anesth* 2020; **34**: 1125-1131 [PMID: 32178954 DOI: 10.1053/j.jvca.2020.02.039]

33 **Zhou F**, Yu T, Du R, Fan G, Liu Y, Liu Z, Xiang J, Wang Y, Song B, Gu X, Guan L, Wei Y, Li H, Wu X, Xu J, Tu S, Zhang Y, Chen H, Cao B. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 2020; **395**: 1054-1062 [PMID: 32171076 DOI: 10.1016/S0140-6736(20)30566-3]

34 **Yang X**, Yu Y, Xu J, Shu H, Xia J, Liu H, Wu Y, Zhang L, Yu Z, Fang M, Yu T, Wang Y, Pan S, Zou X, Yuan S, Shang Y. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Respir Med* 2020; **8**: 475-481 [PMID: 32105632 DOI: 10.1016/S2213-2600(20)30079-5]

35 **Hu X**, Deng Y, Wang J, Li H, Li M, Lu Z. Short term outcome and risk factors for mortality in adults with critical severe acute respiratory syndrome (SARS). *J Huazhong Univ Sci Technolog Med Sci* 2004; **24**: 514-517 [PMID: 15641708 DOI: 10.1007/bf02831124]

36 **Cheng H**, Wang Y, Wang GQ. Organ-protective effect of angiotensin-converting enzyme 2 and its effect on the prognosis of COVID-19. *J Med Virol* 2020; **92**: 726-730 [PMID: 32221983 DOI: 10.1002/jmv.25785]

37 **Yang J**, Zheng Y, Gou X, Pu K, Chen Z, Guo Q, Ji R, Wang H, Wang Y, Zhou Y. Prevalence of comorbidities and its effects in patients infected with SARS-CoV-2: a systematic review and meta-analysis. *Int J Infect Dis* 2020; **94**: 91-95 [PMID: 32173574 DOI: 10.1016/j.ijid.2020.03.017]

38 **Huang I**, Lim MA, Pranata R. Diabetes mellitus is associated with increased mortality and severity of disease in COVID-19 pneumonia - A systematic review, meta-analysis, and meta-regression. *Diabetes Metab Syndr* 2020; **14**: 395-403 [PMID: 32334395 DOI: 10.1016/j.dsx.2020.04.018]

39 **Kumar A**, Arora A, Sharma P, Anikhindi SA, Bansal N, Singla V, Khare S, Srivastava A. Is diabetes mellitus associated with mortality and severity of COVID-19? A meta-analysis. *Diabetes Metab Syndr* 2020; **14**: 535-545 [PMID: 32408118 DOI: 10.1016/j.dsx.2020.04.044]

40 **Odegaard JI**, Chawla A. Connecting type 1 and type 2 diabetes through innate immunity. *Cold Spring Harb Perspect Med* 2012; **2**: a007724 [PMID: 22393536 DOI: 10.1101/cshperspect.a007724]

41 **Wan Y**, Shang J, Graham R, Baric RS, Li F. Receptor Recognition by the Novel Coronavirus from Wuhan: an Analysis Based on Decade-Long Structural Studies of SARS Coronavirus. *J Virol* 2020; **94** [PMID: 31996437 DOI: 10.1128/jvi.00127-20]

42 **Li G**, Hu R, Zhang X. Antihypertensive treatment with ACEI/ARB of patients with COVID-19 complicated by hypertension. *Hypertens Res* 2020; **43**: 588-590 [PMID: 32231220 DOI: 10.1038/s41440-020-0433-1]

43 **Drucker DJ**. Coronavirus Infections and Type 2 Diabetes-Shared Pathways with Therapeutic Implications. *Endocr Rev* 2020; **41** [PMID: 32294179 DOI: 10.1210/endrev/bnaa011]

44 **Strollo R**, Pozzilli P. DPP4 inhibition: Preventing SARS-CoV-2 infection and/or progression of COVID-19? *Diabetes Metab Res Rev* 2020; e3330 [PMID: 32336007 DOI: 10.1002/dmrr.3330]

45 **Guo L**, Wei D, Zhang X, Wu Y, Li Q, Zhou M, Qu J. Clinical Features Predicting Mortality Risk in Patients With Viral Pneumonia: The MuLBSTA Score. *Front Microbiol* 2019; **10**: 2752 [PMID: 31849894 DOI: 10.3389/fmicb.2019.02752]

46 **Fadini GP**, Morieri ML, Longato E, Avogaro A. Prevalence and impact of diabetes among people infected with SARS-CoV-2. *J Endocrinol Invest* 2020; **43**: 867-869 [PMID: 32222956 DOI: 10.1007/s40618-020-01236-2]