ISSN 2222-0682 (online)

World Journal of *Methodology*

World J Methodol 2017 September 26; 7(3): 73-111

Published by Baishideng Publishing Group Inc

World Journal of Methodology

A peer-reviewed, online, open-access journal of methodology

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2016-2019

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Contents

Quarterly Volume 7 Number 3 September 26, 2017

DIAGNOSTIC ADVANCES

MINIREVIEWS

ORIGINAL ARTICLE

Retrospective Study

World Journal of Methodology
Volume 7 Number 3 September 26, 2017

Contents

NAME OF JOURNAL
World Journal of Methodology

ISSN
ISSN 2222-0682 (online)

LAUNCH DATE
September 26, 2011

FREQUENCY
Quarterly

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World Journal of Methodology
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PUBLISHER
Baishideng Publishing Group Inc
7901 Stoneridge Drive,
Suite 501, Pleasanton, CA 94588, USA
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PUBLICATION DATE
September 26, 2017

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http://www.wjgnet.com/bpg/gerinfo/204

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World Journal of Methodology

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World J Methodol 2017 September 26; 7(3): 73-92

DOI: 10.5662/wjm.v7.i3.73

ISSN 2222-0682 (online)

DIAGNOSTIC ADVANCES

Establishing the presence or absence of chronic kidney disease: Uses and limitations of formulas estimating the glomerular filtration rate

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Author contributions: Alaini A, Rondon-Berrios H and Argyropoulos CP reviewed the literature and wrote parts of the report; Malhotra D reviewed the literature and made critical changes in the manuscript; Khitan ZJ added important revisions and constructed figures; Raj DSC made critical additions to the report; Rohrscheib M reviewed the literature and made important additions to this report; Shapiro JI made important revisions and constructed figures; Tzamaloukas AH conceived this report and wrote parts of it.

Conflict-of-interest statement: Dominic SC Raj is supported by RO1 DK073665-01A1, 1U01DK099914-01 and IU01DK09924-01 from the National Institutes of Health. Joseph I Shapiro is supported by HL105649, HL071556 and HL109015 from the National Institutes of Health. The rest of the authors declare no conflicts of interest. This report was not supported by a grant.

Manuscript source: Invited manuscript

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Received: January 31, 2017
Peer-review started: February 6, 2017
First decision: March 6, 2017
Revised: May 17, 2017
Accepted: May 30, 2017
Article in press: May 31, 2017
Published online: September 26, 2017

Abstract

The development of formulas estimating glomerular filtration rate (eGFR) from serum creatinine and cystatin C and accounting for certain variables affecting the production rate of these biomarkers, including ethnicity, gender and age, has led to the current scheme of

diagnosing and staging chronic kidney disease (CKD), which is based on eGFR values and albuminuria. This scheme has been applied extensively in various populations and has led to the current estimates of prevalence of CKD. In addition, this scheme is applied in clinical studies evaluating the risks of CKD and the efficacy of various interventions directed towards improving its course. Disagreements between creatinine-based and cystatin-based eGFR values and between eGFR values and measured GFR have been reported in various cohorts. These disagreements are the consequence of variations in the rate of production and in factors, other than GFR, affecting the rate of removal of creatinine and cystatin C. The disagreements create limitations for all eGFR formulas developed so far. The main limitations are low sensitivity in detecting early CKD in several subjects, *e.g.*, those with hyperfiltration, and poor prediction of the course of CKD. Research efforts in CKD are currently directed towards identification of biomarkers that are better indices of GFR than the current biomarkers and, particularly, biomarkers of early renal tissue injury.

Key words: Chronic kidney disease; Serum creatinine; Creatinine clearance; Creatinine excretion; Estimated glomerular filtration rate; Cystatin C; Renal imaging; Hyperfiltration; Biomarkers of chronic kidney disease

Core tip: Detection of the presence and severity of chronic kidney disease (CKD) is currently based on estimates of glomerular filtration rate based on serum creatinine and cystatin C concentrations plus factors that affect the rate of production of these two biomarkers, and on albuminuria. This scheme has improved detection of CKD and monitoring its course and the effects of therapeutic interventions. However, the scheme's performance in detecting early stages of CKD and in predicting its course is poor, in general. Research in this field is directed towards finding better biomarkers of glomerular filtration rate and, particularly, biomarkers indicating early injury of the renal tissues.

Alaini A, Malhotra D, Rondon-Berrios H, Argyropoulos CP, Khitan ZJ, Raj DSC, Rohrscheib M, Shapiro JI, Tzamaloukas AH. Establishing the presence or absence of chronic kidney disease: Uses and limitations of formulas estimating the glomerular filtration rate. *World J Methodol* 2017; 7(3): 73-92 Available from: URL: http://www.wjgnet.com/2222-0682/full/v7/i3/73.htm DOI: http://dx.doi.org/10.5662/wjm.v7.i3.73

INTRODUCTION

Chronic kidney disease (CKD) has been recognized as a major health problem worldwide with rising incidence, pronounced morbidity and mortality, and rising costs[1]. Early diagnosis, prevention and management of CKD, including treatment of its underlying disease and prevention and treatment of medical conditions for which the presence of CKD is a risk factor, has acquired great importance for health providers[1]. The prevalence of CKD in the United States during the years 1999-2004 was estimated to be equal to 13.1%[2]. Reported prevalence of CKD in various regions of the world varies. For example, recent estimates using different approaches computed a CKD prevalence of 32.5% in a small subject sample in Brazil[3], and 6.7% and 5.8% in larger subject cohorts in Romania and Poland respectively[4,5]. The prevalence of CKD is high in populations with conditions predisposing to it. For example, CKD was detected in 38.6% of individuals with hypertension and a high prevalence of advanced age and obesity[6].

The diagnosis of CKD is associated with important risks of disease in other organs. For example, cardiovascular disease has been recognized as a major risk associated with CKD[4,7]. In a cross sectional study of a large number of subjects with low and middle income in 12 countries, the incidence of CKD was 14.3% overall and 36.1% in high risk individuals, while the awareness of CKD was low and the rate of detection of cardiovascular disease in patients with CKD was also low[8]. Adverse effects of CKD on cardiac function have been reported in patients with heart failure, but preserved ejection fraction[9], diabetics with a doubling of their serum creatinine levels[10], and even healthy kidney transplant donors[11]. In a study from Korea, CKD was associated primarily with increased mortality risks from cardiovascular disease, but also with risks for other morbid conditions including malignancies[12].

Despite the universal recognition of the importance of its early detection, CKD is diagnosed late in several parts of the world[13]. Primary care services have a major role in the diagnosis and management of CKD[6]. Guidelines for detection and management of CKD addressed to primary care medical practitioners have been published for adult[14] and pediatric patients[15]. Education of the public is an important step for early management of CKD. Patients with CKD aware of its importance desire to be informed about its risks and management[16]. Information about CKD is provided to the public in the medical press[17]. Finally, methods for evaluation of the economic impact of CKD[18] and for technological developments addressing the detection and prevention of early stages of CKD[19] are studied.

In this report, we address the current methods for diagnosing CKD. The derivation, uses and limitations of these methods will be detailed. Finally, emerging methods for early diagnosis of CKD will be briefly presented.

CURRENT METHOD FOR DIAGNOSING AND STAGING CKD

Establishing the presence and degree of renal

dysfunction has been based on measuring glomerular filtration rate (GFR). The rational for this is a rough correlation between GFR levels and clinical manifestations of renal failure. Serum creatinine level was the traditional surrogate index of GFR. Currently, the diagnosis and staging of CKD is based on estimated values of GFR (eGFR) and presence of albuminuria[14]. The first development leading to substitution of eGFR for serum creatinine was the computation of the Cockroft-Gault formula[20], which estimates creatinine clearance from serum creatinine, age, body weight and gender and was used extensively in the past for the diagnosis and management of CKD. The Cockroft-Gault formula estimates renal creatinine clearance, not GFR. The differences between these two clearances will be addressed later in this report.

The next important step in the diagnosis and staging of CKD was the development of carefully developed equations computing eGFR based on serum creatinine levels in large prospective studies in which GFR was measured by standard methods. The Modification of Diet in Renal Disease (MDRD) Study was the first one to be used for this purpose[21]. The MDRD formulas for eGFR were subsequently reexpressed using standardized serum creatinine values[22]. In addition to serum creatinine, the determinants of eGFR in the currently used 4-variable MDRD formula include gender, age and race (black or not black). A second 6-variable MDRD formula, which utilizes serum urea nitrogen and albumin levels in addition to the four determinants of eGFR used in the first formula, has similar performance characteristics with the 4-variable formula[22].

A newer set of formulas, the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formulas, was developed by combining data from several studies in which GFR was measured by standard methods[23]. The CKD-EPI formulas, which use essentially the same determinants of eGFR as the MDRD formula, were found to be more accurate than the MDRD formula[23]. This higher accuracy, largely, concerned the range of eGFR values greater than 60 mL/kg per 1.73 m^2. However, the estimates of eGFR by the two formulas do not differ substantially for patients with moderate and advanced CKD, in general. Figures 1-3 show simulated estimates of eGFR by the MDRD and CKD-EPI formulas at different serum creatinine levels and ages in various ethnic groups and genders. Figure 4 shows the comparison of eGFR values obtained from the MDRD and CKD-EPI formulas in subjects enrolled in the NHANES and MDRD studies. In patients with CKD, MDRD and CKD-EPI eGFR values are close in general.

The next step in the development of eGFR formulas was the introduction of cystatin C, which is a small molecular weight (13.3 kDa) protein produced at a steady rate from all nucleated body cells, filtered in the glomeruli and taken up and metabolized by the proximal tubules. Serum cystatin C levels were reported to be superior to serum creatinine levels as indices of GFR. The Chronic Epidemiology Collaboration developed formulas estimating GFR from serum cystatin C levels (the CKD-EPI cystatin C equations) and from both serum cystatin and creatinine levels (the CKD-EPI creatinine-cystatin C equations)[24]. The determinants of eGFR are gender and the level of cystatin C in the CKD-EPI cystatin C equations, and serum cystatin and creatinine, gender and ethnicity (black or other) in the CKD-EPI creatinine-cystatin C equations. eGFR formulas based on serum creatinine or serum creatinine and cystatin C were developed for specific ethnic or age groups, *e.g.*, Chinese[25-27], Japanese[28,29], pediatric[30-33] and elderly[34] populations. Currently, several eGFR equations have been developed or are being developed[35].

Extensive sets of guidelines base the diagnosing and staging of CKD on combinations of eGFR cut-off values and albuminuria[36-39]. In the next section, we will discuss the applications of these guidelines. The limitations of this approach are of importance. In a population study mean measured GFR was higher in men than women but was not different between blacks and whites[40]. The authors of this study concluded that the different incidences of renal disease between blacks and whites were not due to the baseline renal function. The differences between the various equations computing eGFR are also not due to the baseline renal factors, but are keys to understanding the limitations of these equations as will be examined later in this text.

CLINICAL APPLICATIONS OF THE VARIOUS FORMULAS ESTIMATING GFR

Formula comparisons

A number of studies compared the accuracy of various eGFR formulas in various populations and clinical conditions[41-63]. The great majority of these studies concluded that formulas based on serum cystatin C alone or on combined cystatin-creatinine levels are superior to other formulas[41-47,49,50,52-54,56-58,60-63]. One study found greater accuracy with the use of the average creatinine-based and creatinine/cystatin-based eGFR formulas[51]. Another study found that in a Korean population an eGFR formula developed in a Japanese population was superior to other formulas[55]. Two studies found superiority of different eGFR formulas in different patient groups[42,48]. Finally, one study[59] found that the CKD-EPI formula[23] and a Japanese formula for eGFR based on serum creatinine[64] are superior to measured creatinine clearance in monitoring patients receiving cisplatin in high doses.

Uses of eGFR formulas in clinical studies

The older method for the diagnosis of CKD was to compare the serum level of creatinine of a subject to a normal range of creatinine concentrations. The clear advantage of eGFR formulas over this older method is that the formulas allow earlier detection

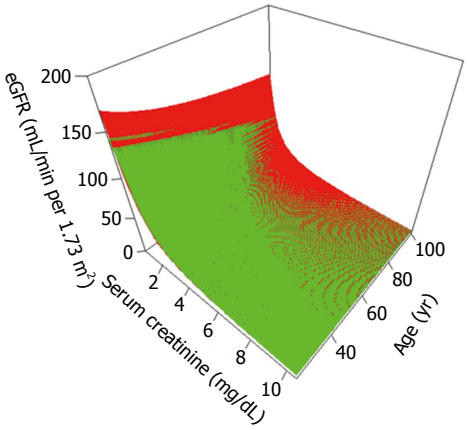

Figure 1 Modification of Diet in Renal Disease[22] and Chronic Kidney Disease Epidemiology Collaboration[23] formulae for estimating glomerular filtration rate fit to variations in serum creatinine (X axis) and age (Y axis) assuming males of Caucasian race. Note that the CKD-EPI formula yields slightly higher eGFR values with higher serum creatinine values and lower age whereas the MDRD formula leads to significantly higher eGFR values at very low serum creatinine values. MDRD: Modification of Diet in Renal Disease; CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration; eGFR: Estimating glomerular filtration rate.

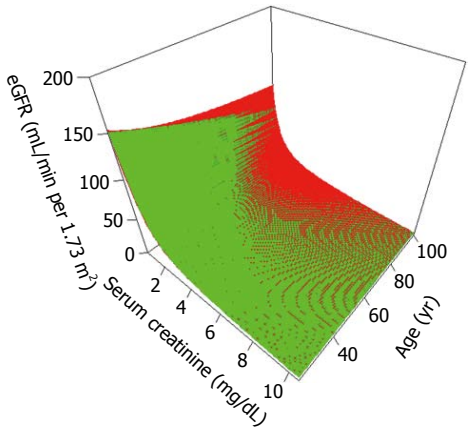

Figure 2 Modification of Diet in Renal Disease[22] and Chronic Kidney Disease Epidemiology Collaboration[23] formulas for estimating glomerular filtration rate fit to variations in serum creatinine (X axis) and age (Y axis) assuming females of Black race. The CKD-EPI formula yields slightly higher eGFR values with higher serum creatinine values and lower age whereas the MDRD formula leads to significantly higher eGFR values at very low serum creatinine values in this population also. MDRD: Modification of Diet in Renal Disease; CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration; eGFR: Estimating glomerular filtration rate.

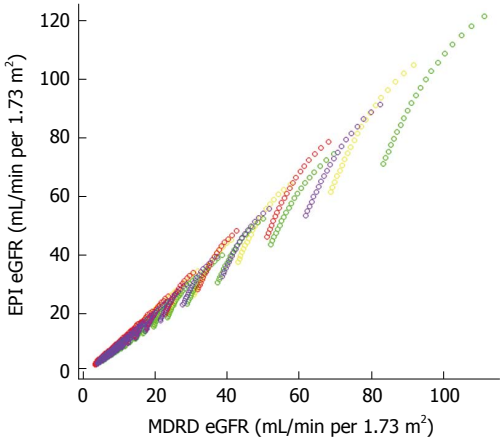

Figure 3 Scatterplot demonstrating close relationships between estimating glomerular filtration rate values calculated by the Chronic Kidney Disease Epidemiology Collaboration formula[23] (Y axis) and the Modification of Diet in Renal Disease formula[22] (X axis). Different colors are used to indicate the races and genders depicted in this figure: Yellow indicates Caucasian males, Green Black males, Red Caucasian females, and Purple Black females. A straight line to fit the data minimizes the least square error with an intercept of -1.03 and a beta coefficient of 1.04 achieving an R^2 value of 0.99. MDRD: Modification of Diet in Renal Disease; CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration; eGFR: Estimating glomerular filtration rate.

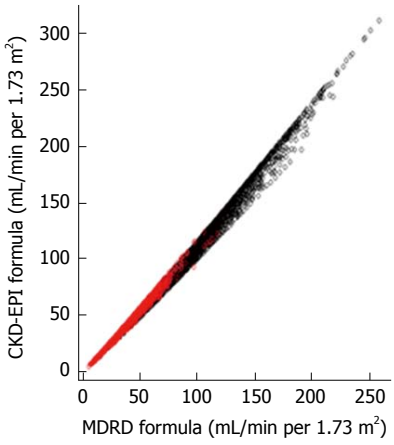

Figure 4 Comparison of estimating glomerular filtration rate values obtained by the Modification of Diet in Renal Disease[22] and Chronic Kidney Disease Epidemiology Collaboration[23] formulas in subjects who were enrolled in the NHANES study (Serum creatinine > 0.4 mg/dL, age ⩾ 20 year) and the MDRD study[22]. MDRD: Modification of Diet in Renal Disease; CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration.

of CKD and more precise following of its course in the early stages of CKD, when large decreases in GFR lead to small rises in serum creatinine. Formulas computing eGFR have been applied in clinical studies for a variety of purposes[43,45,46,57,58,60,65-74]. The Chronic Renal Insufficiency Cohort (CRIC) study, which is studying several aspects of CKD, is using its own eGFR formula based on serum creatinine and cystatin C levels[65]. Estimating the incidence of CKD in populations is one area where eGFR formulas have been useful. Risk prediction in various patient groups with CKD[43,45,46,48,57,58,60,67-69,71], choice and outcome of surgical and medical interventions in patients with CKD[56,72-74], and association of the CKD stage with specific clinical manifestations in various patient groups[66,70] are conditions for which eGFR equations based on cystatin C or on cystatin-creatinine have been shown to provide accuracy. In clinical studies targeting specific end-points of decline in renal function, use of eGFR instead of serum creatinine has the potential of reducing substantially both the required number of participants[75] and the targeted degree of decline in

Table 1 Clinical conditions affecting the accuracy of estimating glomerular filtration rate formulas

Diabetes mellitus
Human immunodeficiency viral infection
Chronic liver disease
Cardiovascular disease
Kidney transplants (recipients and donors)
Sarcopenia
Critical illness
Hereditary disease (*e.g.*, Fabry's)
Obesity

renal function[76].

LIMITATIONS OF THE FORMULAS COMPUTING eGFR

The introduction of the MDRD formula for eGFR[21] and the subsequent development of the current method for detecting and classifying CKD based on eGFR[34,77,78] has enhanced CKD awareness among clinicians and the public and has created new vigor in the study of prevention and management of CKD. Nevertheless, this approach to CKD has significant limitations. This section will discuss sequentially issues with the accuracy of eGFR formulas in clinical states associated with CKD, the analysis in the literature about these issues, the main cause of inaccuracies of the eGFR formulas, and the steps required for establishing the presence or absence of CKD when an eGFR formula computes a value less than 60 mL/min per 1.73 m^2.

Discrepancies of the diagnosis of CKD by eGFR formulas in various clinical states

Discrepancies between various formulas estimating eGFR and between these formulas and measurements of GFR by standard methods have been reported. To illustrate the types of conditions in which eGFR formulas may be inaccurate, we will discuss a few examples of these discrepancies. Table 1 shows clinical conditions in which the accuracy of eGFR formulas has been disputed.

The increasing incidence of diabetes mellitus in many parts of the world has been the major cause of the increasing incidence of CKD. CKD secondary to diabetic nephropathy, which particularly in its early stages may not be associated with albuminuria, especially in type 2 diabetics, has its own diagnostic difficulties[79-92]. Several studies concluded that some creatinine-based eGFR formulas are not as accurate in detecting early CKD as cystatin-based formulas[80-84,86-88,91]. In addition, cystatin-based eGFR formulas were the only ones found to be independent predictors of diabetic complications[83,86] and creatinine-based eGFR formulas did not detect early declines in renal function[89,90]. One study concluded that the prediction of CKD was similar with eGFR formulas calculated by the Cockroft-Gault formula, the MDRD formula and a cystatin-based eGFR formula[79]. A study using inulin clearance to measure GFR concluded that cystatin-based and creatinine-based eGFR formulas have significant inaccuracies in the diagnosis and staging of diabetic CKD[92]. Based on the discrepancies of eGFR formulas, one report proposed the use of one of the standard techniques, iohexol clearance, for evaluation of renal function in type 1 diabetics[85].

Infection with human immunodeficiency virus (HIV) is an important cause of CKD in several parts of the world, for example in South Africa[93]. Proper management of patients infected with HIV requires repeated screening for CKD[94]. Screening for CKD is of great importance for patients treated with nephrotoxic antiretroviral medications[95]. Discrepancies between various eGFR formulas in HIV-infected patients have been reported[96-102]. Extrarenal influences on cystatin C and creatinine metabolism may cause eGFR formula computations differing from the actual renal function[97]. For example, serum cystatin C levels may be elevated in patients with active HIV-infection causing a large underestimation of GFR by cystatin-based eGFR formulas[96,102]. Several eGFR formulas, based on either cystatin C or creatinine, were found to underestimate GFR in one study[98]. Other studies in HIV-infected subjects found superiority of either cystatin-based eGFR formulas[98,100] or creatinine-based formulas[99] in detecting CKD and determining the risks associated with it.

Chronic liver disease is associated with inaccuracy of the eGFR formulas[103-105]. Creatinine-based eGFR formulas systematically overestimate measured GFR in this patient group and the degree of overestimation increases with the severity of liver disease[104]. Cystatin-based eGFR equations are more accurate in these patients[103], but cystatin-based formulas derived in populations with liver disease may prove to have the greater usefulness[105].

Limitations of various eGFR formulas have been reported in subjects with cardiovascular diseases[106-109]. One study calculated similar assessment of cardiovascular risks by the Cockroft-Gault formula and by serum cystatin C level[106]. However, a larger study found substantial differences between eGFR values calculated by creatinine-based and cystatin-based formulas in patients with varying severity of cardiac disease, with creatinine-based eGFR values exceeding the cystatin-based values in most patient categories[107]. Another study concluded that measured GFR, not eGFR formulas, should be used for evaluating the relationship between retinal vasculopathy and renal disease[108]. Differences in the association of creatinine-based and cystatin-based eGFR formulas with non-traditional cardiovascular risk factors (asymmetric and symmetric dimethylarginine blood levels, insulin resistance) in subjects without diagnosed cardiovascular disease, diabetes or CKD was reported in another study[109]. Finally, eGFR formulas were found to be inaccurate in heart transplant recipients[110]. The authors of this last

study proposed the use of measured GFR for assessing kidney function in this patient group.

Discrepancies between eGFR formulas have been found in recipients and donors of kidney transplants. A recent study concluded that creatinine-based and creatinine/cystatin-based eGFR formulas are more accurate than cystatin-based formulas in renal transplant recipients[111]. One study concluded that the MDRD eGFR formula was more accurate in detecting GFR values lower than 60 mL/min per 1.73 m^2 than the CKD-EPI creatinine/cystatin C formula after kidney donation[112], while a second study concluded that creatinine-based eGFR formulas have low accuracy in evaluating renal function in prospective kidney donors[113]. Of note is that the eGFR formulas used in this last study were derived in different ethnic groups. Various problems posed by creatinine-based and cystatin-based eGFR formulas in renal transplant recipients were reviewed by Santos and Martins[114]. Based on these and other studies, United Networks for Organ Sharing (UNOS) require a measured creatinine clearance or GFR for evaluating the renal function of prospective kidney donors. Prospective renal transplant donors illustrate the limitations of eGFR formulas in subjects in whom the need of accuracy in establishing absence of CKD is critical.

Issues with the eGFR formulas were reported in patients with neurological diseases causing sarcopenia[115,116], critically ill patients[117], and patients with hereditary disease[118]. Sarcopenia in subjects with neuromuscular disease is the source of systematic overestimation of GFR by creatinine-based eGFR formulas. Studies have found differences between various eGFR formulas in obese subjects[119-122]. One large study concluded that cystatin-based eGFR formulas are deficient in detecting CKD stage 3 or 4 in obese subjects[119]. In contrast, two smaller studies concluded that creatinine-based equations produce higher eGFR values than cystatin-based formulas and may lead to underestimation of the presence and degree of CKD[120,122]. The finding that sarcopenia is highly prevalent in CKD patients leading to underestimation of the degree of obesity in this patient group[121] provides an explanation for the discrepancies between cystatin-based and creatinine-based eGFR in obese subjects with CKD.

Applications of eGFR formulas in population studies

Several reports have analyzed the performance of various eGFR formulas in different populations[123-133]. Several studies compared various eGFR formulas. A Scandinavian study found a substantially different prevalence of CKD with the use of the MDRD formula than with the use of the Cockroft-Gault formula or of two cystatin-based equations[123]. Similar findings were reported in a study from Uruguay, in which the lowest values of eGFR were found when using the CKD-EPI cystatin C equation, while the CKD-EPI creatinine-cystatin formula computed intermediate eGFR values and the MDRD formula computed the highest values of eGFR[129]. A study in Asian Indians, which also found lower overall eGFR values when using cystatin-based equations, noted that these equations resulted in widely varying eGFR values which affected the classification of CKD[130]. A large study analyzing United States subjects with eGFR determination by the MDRD formula reported that only eGFR values lower than 45 mL/min per 1.73 m^2 yielded a high probability of CKD[132].

In addition, studies in smaller numbers of subjects from various parts of the World compared eGFR formulas and GFR measurements by standard research methods[124-128,131]. Discrepancies between eGFR computed by cystatin-based equations and measured GFR were found in a pediatric Canadian group[124]. Differences between eGFR and measured GFR were also noted in an elderly Chinese group regardless of whether the formula used to compute eGFR was based on cystatin C or not[125]. In a study of Japanese subjects, the creatinine-based Japanese eGFR formula, overestimated GFR in subjects who had poor renal function or were malnourished[126]. In another study in elderly Chinese subjects, there were differences between several eGFR formulas and GFR, with some cystatin-based eGFR equations performing better than other equations[127]. In a study at Mayo Clinic, combined cystatin- and creatinine-based eGFR correlated better with measured GFR than creatinine-based or cystatin-based eGFR values, but creatinine-based eGFR was found to have a better association with most risk factors than the other eGFR values[128]. Another Mayo Clinic study comparing GFR with the CKD-EPI eGFR formulas based on creatinine and cystatin in recipients of organ transplants, patients with known CKD and prospective kidney donors concluded that the performance of various eGFR formulas based on creatinine or cystatin C was affected significantly by the clinical characteristics of the subjects[131]. GFR in these studies was measured by technetium-99m-diethylene-triamine penta-acetic acid [(^{99}m)DTPA][124,125,127], inulin[126], or iothalamate[128,131] clearance.

Formulas computing eGFR, in conjunction with other factors, have been found to be of use in assessing risks associated with CKD. The risk of progression of CKD was recently evaluated in a metaanalysis of studies in large numbers of subjects in North America and other parts of the World performed by the CKD Prognosis Cohort (CKDPC)[133]. This metaanalysis concluded that formulas predicting the risk of progression developed in Canada and including eGFR, age, gender, and albuminuria, plus four serum biochemical values (calcium, phosphate, bicarbonate, and albumin) were accurate in predicting progression, with the proviso that calibration may be needed in certain parts of the World.

Commentaries on creatinine, cystatin C, and eGFR

The issues raised by various eGFR formulas as well as reference methods for measuring GFR have

been addressed in several reports[134-142]. Conditions that may cause false values of creatinine-based or cystatin-based eGFR formulas were addressed in two reviews[134,135]. One study analyzed factors leading to agreement or disagreement between measured creatinine clearance and creatinine-based formulas estimating renal function[136]. The issues faced with the development of risk prediction formulas based on eGFR plus various other factors and with the applications of these formulas were explored in another review[137].

One study found a systematic difference between iothalamate and iohexol, two standard markers of GFR in research studies: The average iothalamate clearance was 15% higher than the average iohexol clearance, while the average creatinine clearance exceeded the corresponding iohexol value by 42%[138]. A related editorial discussed the potential effects of differences in the clearances of standard markers of GFR on the derivation of eGFR formulas[139]. Two reports discussed the use of various indicators of GFR in pediatric[140] and adult[141] populations. Finally, one report presented a complex computer-based program for the diagnosis of CKD based on eGFR and pertinent clinical information[142].

THE MAIN LIMITATION COMMON TO OF ALL eGFR FORMULAS

The section will start with the presentation of three subjects under the care of nephrologists in two hospitals in Albuquerque. These subjects illustrate issues created by eGFR formulas and suggest the proper way to address these issues. Creatinine-based eGFR values were computed by the MDRD[22] and CKD-EPI[23] formulas in all three subjects. In the third subject, cystatin-based and creatinine/cystatin-based eGFR were computed by the CKD-EPI formulas[24]. All eGFR values in these patients are in mL/min per 1.73 m^2.

Illustrative cases

Case 1: A 61-year-old white man with quadriplegia for 25 years following a motor vehicle accident and on hemodialysis for two years transferred to an inpatient spinal cord injury unit in New Mexico from another state. Immediately prior to the first hemodialysis session, his serum creatinine level was 1.27 mg/dL, with eGFR values of 58 by the MDRD formula and 61 by the CKD-EPI formula. Serum creatinine levels ranged between 1.12 and 1.32 mg/dL throughout the dialysis period. The agency overseeing chronic dialysis facilities in New Mexico requested definitive proof of end-stage kidney disease (ESKD). A 48-h urine collection through the permanent indwelling urinary bladder catheter carried by the patient revealed the following values: Volume of collected urine 320 mL; total creatinine content in this urine specimen 62 mg.

Case 2: A 31-year-old white woman had one year after donating a kidney a stable serum creatinine concentration of 1.32 mg/dL, with eGFR values of 47 by the MDRD formula and 53 by the CKD-EPI formula. Urinalysis was repeatedly clean and urine albumin was undetectable. She is dedicated to exercise. Creatinine excretion in a 24-h urine specimen was 1672 mg and serum creatinine collected at the end of the urine collection remained at 1.32 mg/dL. Her body surface area is 1.81 m^2. Calculated renal creatinine clearance was 84 mL/min per 1.73 m^2.

Case 3: A 71-year-old white man with hypertension under excellent control had on repeated testing a serum creatinine level of 1.60 mg/dL, no albuminuria, and ultrasound showing no abnormalities in the renal texture and no post-void residual urinary bladder volume. He is exercising intensely. Serum cystatin C level was 1.0 mg/L. Calculated eGFR was 43 by both the MDRD and CKD-EPI creatinine equations, 57 by the CKD-EPI creatinine-cystatin equation, and 74 by the MDRD-EPI cystatin C equation.

The main limitation of eGFR formulas

The main limitation of the eGFR formulas is significant lack of accuracy in individuals as compared to groups. This causes problems in establishing the presence or absence of early CKD. The inaccuracy is rooted in the method for developing these formulas and the nature of the biomarkers used in them. eGFR formulas are multiple regression equations estimating GFR values using the serum concentration of one or more biomarkers eliminated by glomerular filtration and of other factors that affect the production of the biomarkers. Factors entered in these formulas so far are ethnicity, gender and age. A group of subjects with the same GFR, ethnicity, age and gender will not have the same serum concentration of the biomarker. Instead, they will have a range of concentrations around the mean value for this group, which is the value computed by the eGFR formula. Conversely, the values of GFR will have a range around the mean value provided by the eGFR value. The width of the range determines the standard error of the eGFR estimate provided by the regression equation.

The errors of eGFR formulas derived by regression have been quantified by P30 statistics, *i.e.*, the probability that the measured GFR will differ from eGFR by 30% or less in various cohorts including the cohort that was used for the CKD-EPI formula[143], a cohort of elderly subjects[144] and a cohort of transplant recipients[145]. GFR and eGFR values differ by more than 30% in approximately 20% of the individuals. The origin of this difference lies in the fact that eGFR formulas do not include all the factors that affect the serum concentration of a biomarker or account for changes in the quantitative effects of factors with varying intensity (*e.g.*, degree of sarcopenia). Indeed, development of an eGFR formula accounting for all the

influences on the steady state serum concentration of a biomarker and for quantitative variations of these influences would be an exceedingly difficult task.

The nature of the biomarkers used in eGFR formulas is also a source of differences between eGFR and GFR values. eGFR formulas are applicable only to steady states of biomarkers. In the steady state, the rates of production and removal (the total clearance) of a biomarker are equal and its serum concentration is the fraction production/clearance. An ideal biomarker for GFR would also be exceedingly difficult to find. Such a biomarker should be eliminated exclusively by glomerular filtration and have the same exactly production rate in all subjects with the same age, gender, ethnicity and whatever other factors may be entered in eGFR formulas in the future. In examining whether a biomarker is suitable as an indicator of GFR one should investigate both its production and routes of elimination. Creatinine and cystatin C fail to fulfill the criteria for ideal biomarkers of GFR.

The production of creatinine receives influences from a host of factors other than those entered in the eGFR formulas and its elimination is not only through glomerular filtration. Factors affecting the production of creatinine and not included in the eGFR formulas include level of exercise, diet, particularly red meat ingestion or intake of dietary supplements containing creatine[146-148], neuromuscular diseases leading to loss of muscle mass, and disease states affecting the rate of conversion of creatine to creatinine[149]. Not accounting in the eGFR formulas for factors affecting creatinine production can affect the accuracy of eGFR estimates in individuals as well as cohorts. For example, the reported larger degree of underestimation of GFR by the MDRD formula in healthy individuals than in CKD patients[150] was probably the consequence of higher production rates of creatinine in the healthy individuals.

Creatinine production differences between subjects with and without diabetes mellitus, which is a major cause of CKD, are the reason for the inaccuracies of creatinine-based eGFR formulas discussed earlier. There is evidence suggesting that diabetes affects creatinine production. Serum creatinine levels tend to be low in diabetic individuals[151], reflecting low rate of creatinine production, hyperfiltration (see below) or a combination of the two. Lean body mass, a large part of which is muscle mass, decreases with age rapidly in diabetic subjects[152]. The loss of muscle mass in diabetic subjects is the source of discrepancies between GFR and creatinine-based eGFR formulas[153]. Creatinine excretion is systematically lower in diabetic than non-diabetic subjects with ESKD treated by peritoneal dialysis[154]. A formula developed in a peritoneal dialysis cohort includes diabetes among the predictors of creatinine excretion[155]. Future developments in creatinine-based eGFR formulas should study, and most probably include, diabetes among the factors affecting serum creatinine concentration.

Factors other than GFR affect creatinine excretion. Renal creatinine excretion is not exclusively through glomerular filtration. Tubular secretion contributes a small part, around 15%, of the urinary creatinine at normal GFR values. In glomerulopathic CKD, the fraction of urinary creatinine excreted through tubular excretion increases progressively as GFR decreases and serum creatinine rises[156]. Removal of creatinine through tubular secretion is the source of significant overestimation of GFR by creatinine-based eGFR formulas in CKD. In addition to tubular secretion, creatinine is removed from the body through extrarenal routes, mainly through the gastrointestinal tract. An indirect approach computed an average extrarenal creatinine clearance of 0.042 L/kg per 24-h in males and 0.041 L/kg per 34-h in females with advanced CKD[157]. This approach suggests that progressively larger amounts of creatinine are removed through the extrarenal pathway as serum creatinine rises progressively in worsening CKD. This would cause progressive overestimation of GFR by creatinine-based eGFR formulas.

Sickle cell disease is one condition leading to large differences between renal creatinine clearance and GFR. Creatinine-based eGFR formulas have greatly overestimated true GFR in patients with sickle cell nephropathy. This is related to the supranormal proximal tubular function in subjects with sickle cell disease resulting in enhanced creatinine secretion and various electrolyte disturbances[158,159]. It has been suggested that serum cystatin C and possibly cystatin-based eGFR formulas can be better indicators of renal function in such patients[160] Conditions that affect the extrarenal removal of creatinine need further study.

Several medications affect the production or tubular excretion of creatinine. Table 2 shows medications that affect creatinine production[161,162] or block tubular creatinine secretion[163-168]. In the past, several research studies measured GFR by creatinine clearance with the use of medications blocking tubular creatinine excretion[163]. A great number of medications may induce myopathy leading to varying rates of creatinine production. Drugs induce myopathy by direct myotoxicity (*e.g.*, alcohol, cocaine, glucocorticoids, statins, antimalarial compounds, colchicine, zidovudine), immunological mechanisms causing inflammation (*e.g.*, D-penicillamine), or various indirect mechanisms (*e.g.*, drug-induced coma causing muscle ischemia from compression, diuretic-induced hypokalemia, and drug-induced hyperkinetic syndromes, dystonic states, hyperthermia or neuroleptic malignant syndrome)[169].

Acute drug-induced rhabdomyolysis is manifested by elevation in the serum concentration of muscle enzymes (*e.g.*, creatinine phosphokinase) and leads to acute kidney injury in some instances, in addition to a rise in serum creatinine due to overproduction. In chronic drug-induced myopathy causing increased creatinine production, however, serum levels of muscle enzymes may not be elevated[169]. In subjects

Table 2 Drugs raising serum creatinine concentration

Drug	Ref.
Drugs enhancing creatinine production	
Fenofibrate	[161]
Vitamin D receptor activators	[162]
Drugs inhibiting tubular creatinine secretion	
Cimetidine	[163]
Cobicistat	[164]
Dronedarone	[165]
Pyrimethamine	[166]
Salicylates	[167]
Trimethoprim	[168]

with advanced CKD, for example ESKD patients treated by peritoneal dialysis, increased creatinine production secondary to drugs can cause large rises in serum creatinine concentration and excretion without a change in creatinine clearance or in the serum levels of muscle enzymes[170]. Finally, errors in serum creatinine values are caused by interference of various substances, endogenous or exogenous, with the creatinine assay. This issue was more common with the older method of measuring creatinine concentration in biological fluids by the non-specific Jaffe colorimetric method[171]. However, even the newer specific enzymatic methods receive interference from other substances, including dopamine, ascorbate[171], the analgesic dipyrone (metamizol), N-acetylcysteine and other substances.

Like creatinine levels, serum cystatin C levels receive influences independent of GFR[172-183]. Changes in renal and extrarenal function and in the production of cystatin C may affect its serum level. As noted cystatin C is filtered in the glomeruli and then reabsorbed and catabolized in the proximal tubules. Its urinary excretion is a small fraction of its filtered load. It has been postulated that tubulointerstitial disease damaging the integrity of the tubular barrier may lead to back leak of cystatin C into the peritubular blood capillaries and increase in serum cystatin C levels[172]. A change in tubular handling of cystatin C was reported in children with nephrotic syndrome who exhibited significant rises in the urinary excretion of the compound at times of heavy proteinuria[175]. Potential influences of renal tubular dysfunction on cystatin C serum levels will require further study.

Factors affecting the extrarenal clearance and production of cystatin C have not been studied adequately. Indirect methods to quantitate these influences have been proposed[177]. Increases in the rate of production and the serum levels of cystatin C have been reported with the use of corticosteroids[173], and in subjects with obesity[180], large lean body mass[178], hyperthyroidism[178,183], elevated serum triglyceride levels[181], and after coffee consumption[182]. Two reports studied by multivariable statistical analysis factors affecting serum cystatin C levels independently of GFR[174,179]. Older age, male gender, large weight and height, current cigarette smoking and higher C-reactive protein levels were associated with higher serum cystatin C levels in a study from the Netherlands[174]. A study pooling data from three large research studies identified younger age, male gender, diabetes mellitus, high levels of C-reactive protein, high white blood cell count and low levels of serum albumin as independent factors associated with high levels of cystatin C[179]. Race had also a small independent effect on cystatin C in this last study. Finally, potential interferences with the measurement of cystatin C have not been adequately studied.

How to proceed when a low eGFR value suggest CKD in an individual

Serum creatinine is routinely measured for surveillance of the renal function, while cystatin C is not a routine blood test. Currently, the initial step for diagnosing and staging CKD is based on determination of serum creatinine and albuminuria. Creatinine-based eGFR values are of paramount importance in this process[39]. The rate of creatinine production is the cause of questioning the accuracy of eGFR in the great majority of subjects. The suggested next step in the evaluation of individuals for whom there are questions about the accuracy of creatinine-based eGFR is to measure serum cystatin C and compute cystatin-based and creatinine/cystatin- based eGFR values[39]. This approach, which was based on the finding that the effect of muscle mass on serum cystatin C levels is small[179], may confirm the presence of CKD in subjects in whom the cystatin-based eGFR values agree with the creatinine-based values, but will create new problems if the various eGFR values disagree. The subject reported in case 3 above illustrates this problem.

A different approach for confirming or rejecting a questionable creatinine-based eGFR value is required. The hypothesis that an unusual rate of creatinine production led to an unusual serum creatinine level is addressed directly by measuring creatinine excretion rate[184]. Cases 1 and 2 of this report illustrate this point. As noted, candidates for living kidney donation are a group of subjects who should also have their creatinine clearance determined. Differences between estimates of eGFR and estimates of creatinine clearance by the Cockroft-Gault formula affecting significantly the dosing of potentially toxic drugs have been reported[185]. Dosing of drugs may require measurement of creatinine clearance in selected cases. In addition to clarifying the information provided by creatinine-based eGFR, determination of creatinine excretion has prognostic significance. Low urinary creatinine excretion levels in CKD patients are associated with adverse outcomes[186].

Establishing the presence or absence of CKD from creatinine clearance also has drawbacks. Urine collection and storage for 24 h is a demanding task. Errors in the timing of urine specimens and presence of obstructive urinary tract disease are sources of

inaccuracy of urine collection. Detailed explanation of the importance of accurate urine collection and detailed instruction about the timing of this procedure are imperative and minimize collection errors. Motivation of individuals with questionable eGFR values is important. Subjects with low creatinine-based eGFR values and suggestion of large muscle mass are usually very motivated to know whether they have CKD or not. Evaluation for urinary obstruction should be considered a necessary step of the diagnosis of CKD.

A third drawback of the measured creatinine clearance is the systematic overestimation of GFR because of tubular secretion of creatinine. This may become significant when the value of the measured creatinine clearance is above, but close to 60 mL/min per 1.73 m^2. In glomerulopathic subjects with normal GFR values, creatinine clearance exceeded by 16%, on the average, GFR measured by inulin clearance[156]. We suggest that creatinine clearance values less than 72 mL/min per 1.73 m^2, that is values exceeding 60 mL/min per 1.73 m^2 by less than 20%, should call for further investigation. Cystatin C measurements may improve the accuracy of diagnosis of CKD in this last group of subjects, but will require further studying.

As noted, careful history taking about conditions predisposing to CKD is very important in establishing the diagnosis of CKD[142]. Imaging techniques are also of help. Size and texture of the kidneys and features of obstructive disease of the urinary tract are routinely investigated by traditional ultrasonographic techniques. Addition of color Doppler and spectral Doppler to conventional ultrasonography allows detailed investigation of the renal circulation. Circulatory indices derived from these newer techniques, including the resistivity index and the strain index, as well as evaluation of renal fibrosis by elastography, can provide valuable assistance in the diagnosis of CKD[187-189]. Measurement of GFR by nuclear scanning[190,191] is another imaging technique assisting the early detection of CKD in vulnerable patient groups, *e.g.*, renal transplant recipients. Other imaging techniques applied in the diagnosis of renal diseases (*e.g.*, radiography, computed tomography, nuclear magnetic resonance methods) have limited value in the detection of CKD. Despite the proliferation of studies evaluating various biomarkers as indicators of specific renal histology, renal biopsy remains the gold standard of the histologic diagnosis of renal disease[192]. The role of renal biopsy in establishing the presence of CKD, however, is questionable.

FUTURE DEVELOPMENTS

Two types of findings have generated questions about the use of either measured GFR or eGFR formulas for the diagnosis and classification of CKD. The first issue was the varying association between the serum levels of several small molecular weight substances classified as uremic toxins and eGFR values calculated by several formulas in subjects with CKD stages 2 to 5[193]. This finding, which was attributed to varying effects on the serum concentration of uremic solutes of factors other than GFR, including tubular handling, extrarenal removal and production[194] provides a stimulus for searching for new biomarkers of eGFR better associated with various uremic toxins, but does not eliminate the current principle of diagnosis and staging CKD based on GFR. Based on the conclusions of the last report[194], it is possible that other factors have significant effects on the serum concentration of uremic toxins independently or in addition to the presence and stage of CKD. Findings of elevated serum levels of "uremic" toxins in subjects without clinical features of CKD and normal levels of GFR measured by standard methods would provide support to this last postulate.

Hyperfiltration is the second category of findings creating questions about using eGFR or GFR to diagnose and stage CKD. Hyperfiltration has been extensively investigated in subjects with type 1 or type 2 diabetes mellitus in whom it is considered an important factor in the initiation and progression of diabetic nephropathy[195]. Subsequently, hyperfiltration was reported in a variety of clinical conditions including obesity, hypertension, metabolic syndrome, smoking, sickle cell disease, thalassemia, IgA nephropathy, reflux nephropathy, kidney donors, transplanted kidneys, cirrhosis, pregnancy, lead poisoning, autosomal dominant polycystic kidney disease, primary aldosteronism, nephropathy from Puumala hantavirus, and apparently healthy subjects to whom it confers a risk of CKD and hypertension[196]. It is of interest that intense exercise can decrease the prevalence of hyperfiltration in the general population[197].

Establishing the presence of hyperfiltration is of great importance. A review of studies on hyperfiltration disclosed the use of a variety of methods for measuring or estimating GFR and a variety of GFR or eGFR cut-off values defining hyperfiltration[198]. Hyperfiltration is defined as either supernormal GFR or increased filtration fraction (GFR over renal plasma flow), which is normally around 0.20. Elevated glomerular capillary hydrostatic pressure, with or without an elevation in the renal plasma flow, leads to hyperfiltration[195]. Subjects with hyperfiltration on the setting of increased renal plasma flow, for example a subset of patients with early diabetic nephropathy, have supranormal GFR. A few studies of hyperfiltration have measured both GFR and renal plasma flow. In addition to its accuracy in establishing presence or absence of hyperfiltration, this method allows detection of hyperfiltration in subjects with established CKD and low GFR values.

Hyperfiltration creates two major problems with the diagnosis of CKD. The first problem is a documented lack of accuracy of various eGFR formulas in subjects with hyperfiltration[199,200]. The second problem is even more serious and cannot be corrected by finding new biomarkers providing accurate eGFR formulas in

Table 3 New biomarkers for chronic kidney disease

Biomarker	Ref.
Biomarkers for GFR	
Symmetrical dimethylarginine	[212,213]
Beta-trace protein	[214,216,217]
β2-microglobulin	[215-218]
Galectin-3	[219]
Biomarkers for injury of renal tissue	
MicroRNA	[220,221]
Soluble urokinase-type plasminogen activator receptor	[209,222,223]
Proteomics	[224,225]
Gelatinase-associated lipocalin	[226,227]

GFR: Glomerular filtration rate.

subjects with hyperfiltration. Even if such biomarkers are found in the future, subjects with hyperfiltration, early stages of CKD, absence of albuminuria and GFR in the normal range will be misclassified as not having CKD by the current scheme. Elderly subjects with apparently normal renal function, but with loss of nephrons and hypertrophy of the remaining nephrons[201-203] are one such group. Detection of CKD in these subjects requires an approach other than measurement or estimation of GFR.

The limitations of the available tools for diagnosing and staging CKD have complicated the development of accurate models for early detection of CKD and prediction of its progression. One approach that has been investigated is the measurement of the renal functional reserve (RFR)[204]. RFR, the temporary increase in renal blood flow and GFR after a standardized heavy protein meal, is a homeostatic mechanism prominent in carnivores. Healthy humans exhibit a less pronounced, but quite large RFR after a protein meal[204]. Certain studies suggested that measurement of renal functional reserve may be a useful method in detecting early CKD. In one study, vasculopathic patients with normal GFR and absent RFR by GFR measurement developed within two years a significant decrease in GFR[205]. In another study, the magnitude of RFR decreased progressively with higher stages of CKD[206]. Doppler ultrasonography can assist in the evaluation of RFR[207,208]. However, conflicting RFR findings have been reported in several studies of patients with CKD. Whether RFR measurement can provide a useful method for establishing the presence or absence of early CKD is not clear currently.

Another approach to the early detection of CKD is acquiring strength. Recent reports have stressed the need for new biomarkers that can enhance the accuracy of CKD detection[137,209,210]. Table 3 shows biomarkers evaluated in CKD. Current practices utilize two categories of biomarkers for CKD, those that estimate GFR and those that indicate damage to a specific renal function (albuminuria). Similarly, the search for new biomarkers has two directions, indicators of GFR which are routinely assayed in the serum[211-219] and indicators of specific types of kidney injury which are assayed in the urine or serum[220-227].

Demographic and clinical associations of creatinine and newer GFR biomarkers, including β-trace protein, β2-microglobulin, and cystatin C, differ[216]. In specific patient groups, newer GFR biomarkers may offer specific advantages. For example, cystatin C and β2-microglobulin are more sensitive indicators of decreased GFR than creatinine in critically ill children[215], while β-trace protein and β2-microglobulin serum levels may identify additional risk factors in patients with CKD[218]. However, the role of these newer biomarkers in establishing the diagnosis of CKD will need further investigation. For example, eGFR estimates from equations based on β-trace protein or β2-microglobulin were found to be less accurate that the creatinine-based or creatinine/cystatin-based CKD EPI equations[217].

In patients with ESKD, serum levels of biomarkers eliminated poorly by dialytic techniques can provide more accurate estimates of residual GFR than the estimation as the average of renal creatinine and urea clearances[228]. Residual renal clearance may be estimated by pre-dialysis β2-microglobulin or β-trace protein concentrations in patients receiving high flux hemodialysis or hemodiafiltration[229,230]. A preliminary report identified four metabolites, including acetyl-threonine, pseudouridine, acetyl-alanine, and myo-inositol with higher correlation values with measured GFR than serum creatinine levels[231]. eGFR formulas containing multiple metabolites appear to be more accurate than creatinine-based formulas in estimating GFR[232-234]. Despite the promise that this strategy holds, it should be noted that even system-biology combined markers derived from a rational process only moderately improve performance relative to clinical and standard laboratory evaluations. Also, the cost of measuring these biomarkers *vs* measuring GFR by a standard method is an issue that will be raised.

Regardless of the potential advantages of eGFR estimates from new biomarkers, these estimates will have some of the limitations discussed above. The main progress in the diagnosis of CKD and the prediction of its course is expected to result from introduction of biomarkers of renal tissue damage in clinical practice[221]. Note that biomarkers of renal tissue injury assayed in serum may also provide estimates of GFR and have the potential of providing clues about the histologic diagnosis of CKD[235] and combination of clinical predictors and biomarkers can predict progression of CKD in specific patient groups, *e.g*., subjects with type 2 diabetes mellitus[236].

CONCLUSION

The diagnosis and staging of CKD based on estimates of GFR from serum creatinine and cystatin C concentrations represents a major step in the diagnosis of CKD and in following its course, but has significant limitations. The main limitations of this methodology are its low discriminatory power in establishing the

presence or absence of early CKD in individuals and its unsatisfactory performance in predicting the course of CKD. The direction of Research in this field is currently towards identifying new biomarkers that either are superior indicators of GFR, or indicate early injury of the renal tissue. The last group of biomarkers has the potential of leading to improved early detection of CKD.

ACKNOWLEDGMENTS

The authors express their appreciation to the Research Service of the Raymond G. Murphy VA Medical Center for its support of this work.

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P- Reviewer: Bellomo G, Kiykim A, Raikou VD, Zuo L
S- Editor: Ji FF **L- Editor**: A **E- Editor**: Li D

World Journal of Methodology

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

World J Methodol 2017 September 26; 7(3): 93-100

DOI: 10.5662/wjm.v7.i3.93

ISSN 2222-0682 (online)

DIAGNOSTIC ADVANCES

Accurate diagnosis of prenatal cleft lip/palate by understanding the embryology

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Author contributions: All authors contributed to this paper.

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

Manuscript source: Invited manuscript

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Received: December 15, 2016
Peer-review started: December 19, 2016
First decision: March 28, 2017
Revised: May 15, 2017
Accepted: May 30, 2017
Article in press: May 31, 2017
Published online: September 26, 2017

Abstract

Cleft lip with or without cleft palate (CP) is one of the most common congenital malformations. Ultrasonographers involved in the routine 20-wk ultrasound screening could encounter these malformations. The face and palate develop in a very characteristic way. For ultrasonographers involved in screening these patients it is crucial to have a thorough understanding of the embryology of the face. This could help them to make a more accurate diagnosis and save time during the ultrasound. Subsequently, the current postnatal classification will be discussed to facilitate the communication with the CP teams.

Key words: Cleft lip; Cleft palate; Embryology face; Orofacial clefts; Ultrasound

Core tip: Cleft lip/palate is a very common craniofacial malformation. Currently a thorough ultrasound examination during the 20-wk ultrasound is performed to exclude an oral cleft of the face. This study provides important embryological information to facilitate the ultrasonographer in making an accurate diagnosis and safe time during the ultrasound. Subsequently, the current postnatal classification will be discussed to facilitate the communication with the cleft palate teams.

Smarius B, Loozen C, Manten W, Bekker M, Pistorius L, Breugem C. Accurate diagnosis of prenatal cleft lip/palate by understanding the embryology. *World J Methodol* 2017; 7(3): 93-100 Available from: URL: http://www.wjgnet.com/2222-0682/full/v7/i3/93.htm DOI: http://dx.doi.org/10.5662/wjm.v7.i3.93

INTRODUCTION

Orofacial clefts (OFCs) are common craniofacial malformations. Cleft lip (CL) with or without cleft palate (CL/P) occur more commonly in males, while 1:1000 Caucasians, 2:1000 Asians, and 0.3:1000 Africans are affected[1]. However, isolated cleft palate (CP) is more common in females and an equal incidence of 0.4:1000 live born is encountered in all races[1]. Although the distribution for clefts differs per region it is estimated to be 20%-25% CL, 40%-50% CLP and 30%-35% CP. Clefts occur in a ratio of 6:3:1 unilateral left, unilateral right, and bilateral[2]. The etiology of OFCs is complex and believed to be multifactorial, representing an interaction between genetics and environment during a critical stage of development[3]. Recently several genes causing CL and palate have been discovered. The nature and function of these genes vary widely, illustrating high complexity within the craniofacial developmental pathways[4-6]. The interested reader is referred to comprehensive studies that focus specifically on these genes.

In different countries routine ultrasound screening in pregnancy does not consistently include screening for facial clefts. However, the increased use of transabdominal ultrasound (3D) certainly leads to an increased frequency of oral clefts being diagnosed antenatally[7]. There are few articles that focus on different ultrasound approaches to visualize the palate and lips both 2D and 3D[8-10].

As oral clefts typically occur in facial areas where the normal embryological fusion of structures did not occur, knowledge of the embryological background could aid the ultrasonographer to understand and more accurately diagnose these clefts.

The aim of this review is to familiarize the ultrasonographer with the embryology of the face, which will subsequently aid in more accurate diagnosis of the extent of the facial cleft. For a more extensive overview the reader is referred to textbooks[11-13]. The different classifications systems of clefts are also summarized. This might facilitate communication between the ultrasonographer and the CP team after birth.

DEVELOPMENT OF THE LIP

The basic morphology of the face is established between the 4th and 10th week after conception. Upper lip formation commences at 24 d postconception and is completed by 37 d[11-13]. At five weeks' gestation, when the embryo is 3 mm long, the ectoderm in the vicinity of the neural plate folds on itself to form the neural tube. Special neural crest cells of ectodermal origin differentiate to form a special ectomesenchyme. The ectomesenchyme migrates over and around the head and participates in the formation of five facial prominences that surround the primitive oral cavity: The frontonasal prominence, the paired maxillary prominences and the paired mandibular prominences.

The frontonasal prominence develops in the midline over the brain. During the 5th week of embryogenesis the nasal component of the frontonasal prominence forms bilateral two ectodermal thickenings, the nasal placodes (Figure 1)[13]. Each nasal placode invaginates to form an oval nasal pit and divides the frontonasal prominence into a medial and lateral nasal process. During the 6th week, the two medial nasal processes fuse and gives rise to the midline of the nose, medial part of the upper lip, philtrum, incisor teeth and the primary palate. The primary palate is the part of the palate that is located ventrally to the foramen incisivum, while the secondary palate is the part located dorsally to the foramen incisivum. The lateral nasal process subsequently forms the nasal alae and alar base.

During the 6th week the maxillary processes on each side of the mouth grow forward and merge with the medial nasal processes that lead to the formation of the lateral upper lip, the majority of the maxilla and the secondary palate. The mandibular prominences give rise to the mandible and lower lip. The fusion of the facial swellings occurs between the 4th-6th weeks postconception. Failure of fusion between any of the facial swellings results in facial clefts and can occur either unilaterally or bilaterally and typically happens at the junction of the lateral incisor and the first premolar teeth.

In patients with mild CL defects the cleft could be limited to a notch in the vermillion border of the lip that probably represents a failure of localized growth of the medial nasal process. In more severe defects, the cleft runs through all the lip structures and completely separates the lateral lip from the philtrum and nasal cavity. These clefts are caused by failure of fusion between the medial nasal process and the maxillary prominence. The depth of the cleft may vary from the soft tissue of the lip to a complete cleft of the maxillary bone. The normal palate fusion process starts at the foramen incisivum and subsequently closes in a posterior direction. The actual lip fusion starts cranially and subsequently closes in a caudal direction.

Oblique OFCs can also involve the side of the face and even involve the orbit. These clefts comprise less than 1% of facial clefts and can be classified according to Tessier's anatomical classification[14]. Midline clefting syndromes can be divided into two groups: The premaxilla agenesis-holoproencephaly syndrome and frontonasal-median cleft syndrome[15]. Midline clefts arise due to incomplete merging of the median nasal prominences that form the inter-maxillary segment. The premaxilla agenesis-holoproencephaly syndrome (Demyer sequence) has a frontonasal deformity associated with hypotelorism, holoprosencephaly and facial deformity ranging from cyclopia to midline facial cleft with pre-maxillary ageneses. The median cleft face syndrome is often associated with a nasal deformity and hypertelorism usually either with no or little brain deformity (corpus callosum agenesis). In these cases

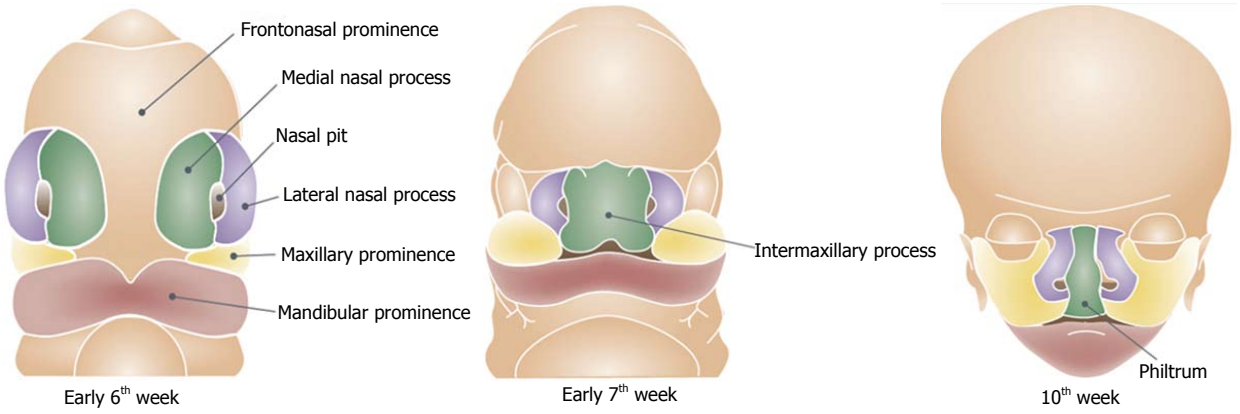

Figure 1 Development of the lip.

surgical reconstruction is feasible due to the probability of normal life expectancy.

DEVELOPMENT OF THE PALATE

Palatogenesis begins at the end of the 5th week and the development of the palate is not completed until the 12th week postconception[11-13]. The palate develops from two primordia: The primary and the secondary palate. The most important cell types in palate development are the neural crest-derived palatal mesenchyme, ectoderm-derived epithelial lining and the most apical layer composed of periderm cells[16]. The soft palate also includes the cranial paraxial mesoderm derived myogenic cells.

The primary palate is formed by merging of the medial nasal prominences during the 6th week and gives rise to the four central incisors and extends to the foramen incisivum.

The secondary palate that separates the nasal cavity from the oral cavity is the primordium of the hard and the soft palate and is formed by the fusion of neural crest mesenchyme that lies within the maxillary primordia. The development of the secondary palate starts with the outgrowth of two palatine shelves from the maxillary process that extend vertically on either side of the tongue. As the mandible grows downward and forward, the tongue's position descends. The palatal shelves subsequently rotate to a horizontal position dorsal to the tongue and then undergo intramembranous ossification to form the palatine process of the maxilla and the palatine bone. The transition from vertical to horizontal position happens in the eight week postconception and is completed, incredibly, in only some hours. There is considerable sex difference in the timing of palatal closure. Shelf elevation and fusion begin a few days earlier in males than in females[13]. Just as the formation of the lip, the subsequent fusion process is an incredibly complex process. Before fusion the palatal shelves are two cell layers thick. The outer layer is sloughed off (by apoptosis), leaving only a basal epithelial layer which composes the medial edge of each palatal shelf. The shelves grow towards each other in the midline and approximate to form the midline epithelial seam. The seam subsequently degenerates, leading to mesenchymal confluence between the two palatal shelves. The fusion process of the shelves starts immediately behind the foramen incisivum and extends dorsally to close the palate like a "zip" (Figure 2). At the same time fusion with the nasal septum and the primary palate occurs. Gradually, bone extends from the palatine process of the maxilla and the palatine bone into the palatal shelves to form the hard palate. The posterior parts do not become ossified and extend posteriorly and fuse to form the soft palate, including the uvula[13].

If the fusion process of the palate which occurs between the 9th and 12th week of gestation, is disrupted by either genetic, mechanical or teratogenic factors, a cleft of secondary palate results. Because the secondary palate closes from the foramen incisivum in a posterior(dorsal) direction, it is not possible for the palate to be open just posterior to the foramen incisivum in the hard palate and then subsequently fuse again in the soft palate part. If the initial process of fusion is defect the rest of the fusion process will not take place. This means that an intact soft palate implies the presence of an intact hard palate. Only a handful of cases have been described where "fenestrations" have been found in the midline of the palate seam[17]. These cases have been attributed to trauma and not due to a defective fusion process. Especially the submucous cleft could be vulnerable[17]. However, some mouse models do suggest that initial contact is made in the middle-anterior region with fusion proceeding in both anterior and posterior directions[17].

Abnormality of the mandible appears to have a related cause with CP. Hypoplasia of the mandible (micrognathia) interferes with descent of the tongue and positions the tongue superiorly between the two palatal shelves. This causes mechanical disruption of palatal closure and could result in a CP. Micrognathia could be associated with Robin sequence. This sequence or phenomenon consisting of a triad of micrognathia, glossoptosis and breathing problems often involves an associated CP[18]. This condition could be associated

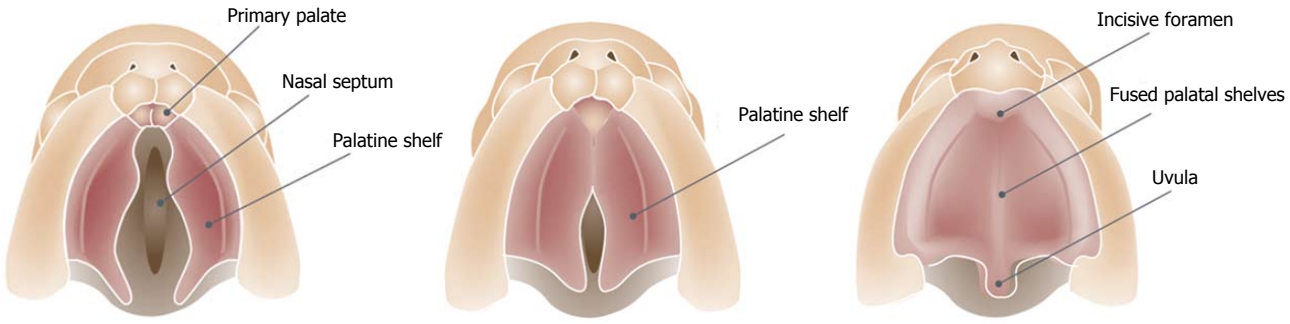

Figure 2 Caudal view of the fusion process of the palatal shelves.

with life threatening breathing problems postnatal and is often associated with other anomalies such as heart defects[19].

IMPLICATIONS FOR DIAGNOSING AN OROFACIAL CLEFT

Fusion between the two maxillary processes differs on a molecular level from the fusion between the medial nasal process and the maxillary process. Together with the epidemiological differences this supports the view that CL/P (CL with or without CP) and isolated CP are two different entities[6-13].

Postnatal the prevalence of associated anomalies is lowest in CL and ranges from 7.6%-41.4%[6-13]. Data from postnatal studies show that concerning CLP the frequency is higher and ranges from 21.1%-61.2%. CP is the category most frequent associated with additional congenital anomalies and the prevalence of associated malformations with CP ranges from 22.2%-78.3%. Antenatally isolated CP is usually not diagnosed and associated abnormalities are reported in 39.1%-66.0% of foetuses with CLP[20,21]. This high percentage probably reflects many different factors: The intra-uterine lethality of associated syndromes such as trisomy 13 or 18, pregnancy termination for severe malformations and the greater likelihood of diagnosing CL/P in the case of more associate abnormalities, especially if evaluation of the lip does not constitute part of routine screening.

It is becoming more frequent to use the trans-abdominal ultrasound screening during the second trimester of pregnancy to evaluate the face. Evaluation of the upper lip for possible CL/P is an optional element and has a sensitivity of 88% for detecting CL/P[21]. However the overall sensitivity for OFCs is lower because the prenatal detection rate of CP is only 0%-1.4%[21]. The very low detection rate of CP demonstrates that there are no satisfactory sonographic indicators of an isolated CP that might be one of the reasons why the palate is often not visualized during the ultrasound screening. Yet, the ultrasonographer should be aware that micrognathia could be associated with a CP. Subsequently, when the ultrasonographer encounters other malformations (such as heart defects) with the micrognathia, evaluation of the palate is mandatory.

SPECTRUM OF OFCS

Some forms of CL only have a small indentation in the vermillion. This "forme fruste" with a small notch within the borders of the vermillion and a band of fibrous tissue running from the edge of the red lip to the nostril floor or a deformity of the nasal ala on the side of the notch, is unlikely be diagnosed by ultrasound. When CL is seen on 2D or 3D ultrasound, the position of the alar base (nostrils) can help to determine whether the alveolar ridge or palate is involved as well. An isolated incomplete CL without a maxillary or palatal defect will appear as a linear defect running from the lip towards the nasal floor (Figure 3A). In complete CL the lip defect could be well visualized, although the nose distortion is likely to be minimal (Figure 3B). In complete bilateral CL the maxilla is intact (there is no maxillary protrusion) and the alar bases or nostrils are symmetrical. Recently we have demonstrated that with a normal maxilla-nasion-maxilla angle, it was unlikely to find a cleft that included the alveolus[22]. A complete CL with alveolar ridge involvement but without involvement of the primary palate is called an incomplete CL. Incomplete involvement of the alveolar ridge usually does not substantially change the position of the alar base. A complete CL is diagnosed as involvement of the complete lip, alveolar ridge and primary palate. A complete CLP includes the lip, alveolus, primary palate and whole secondary palate from foramen incisivum to include the uvula. In a complete CLP the lip involvement is relatively easy to detect by ultrasound, while the alar base is characteristically lateralized away from the cleft (Figures 3C and 4). In cases with bilateral CLP there is a protrusion of the maxillary process that is visible as an echogenic structure below the alar base (Figure 3D and E). This protrusion is not commonly seen in cases without a secondary CP and could help the ultrasonographer when confronted with a bilateral CL deformity. An important message for sonographers is that cleft alveolus does not necessarily equal CP. Lateralization of the alar base could help the

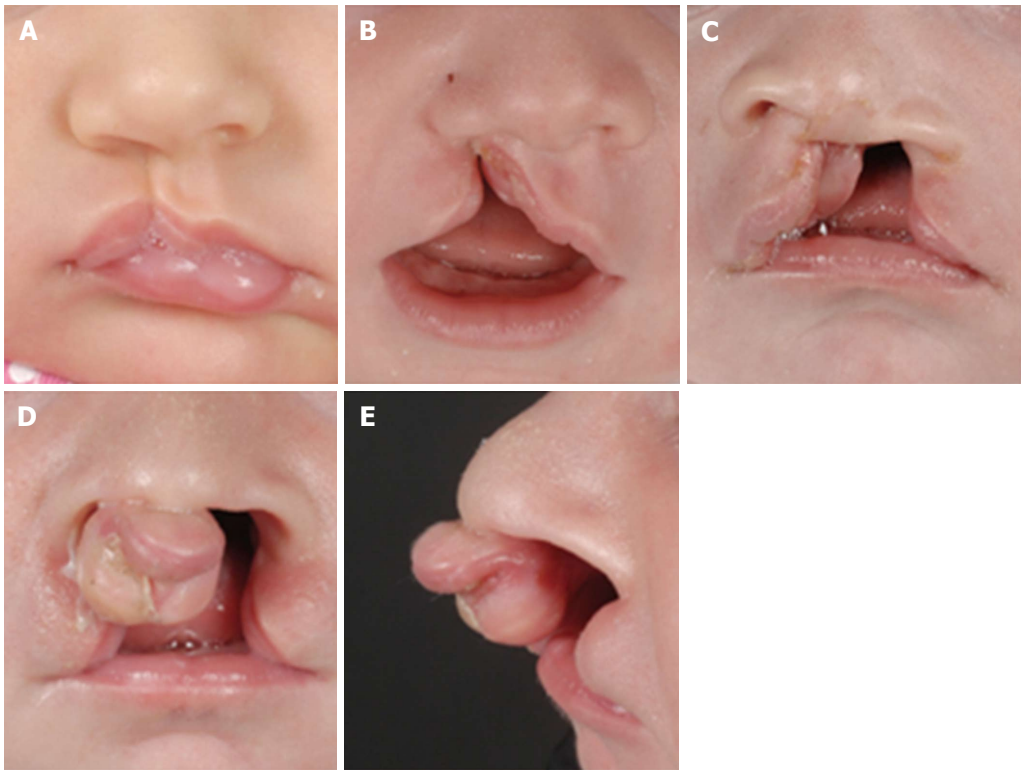

Figure 3 Different types of cleft lips. A: Unilateral microform cleft lip; B: Unilateral cleft lip and alveolus; C: Unilateral cleft lip and palate; D: Bilateral cleft lip and palate with protrusion of the intermaxillary process; E: Lateral view of bilateral cleft lip and palate.

ultrasonographer to diagnose complete CLP patients where the visualisation of the CP is difficult. Different techniques such as the oblique-face or flipped-face view make better 3-D visualization of the alar base[8,9,10,23].

Unless other anomalies alert the ultrasonographer to the possibility, isolated CP is usually not diagnosed prenatally whereas the palate has a dome shaped structure and is surrounded by osseous structures making it difficult to visualize. The fact that the embryological fusion of the secondary palate starts from the foramen incisivum and proceeds dorsally is of importance during ultrasonography. An unremarkable uvula implies the presence of an intact palate. If the uvula is deformed or absent this might indicate a defect in the palate. The uvula can be visualized by ultrasound and the echo pattern of a normal uvula is typical and strongly resembles an "equals sign" (Figure 5)[16]. It is important to realize that the vocal cords also have "two lines" and could resemble the "equal sign of the palate". However, the vocal cords are located lower than the soft palate. If the equals sign cannot be seen, CP cannot be ruled out and should be further examined by imaging the soft palate in a median sagittal section to visualize the palate structure in more detail. Larger prospective studies are needed to confirm this hypothesis. Implementation of the equals sign technique may improve the prenatal detection rate of isolated CP[16,24]. Moreover if the ultrasonographer cannot visualise the middle part of the secondary palate (*e.g.*, obstruction of the tongue) but the "equals sign" is visible, it is suggestive of an intact palate. This could subsequently save the ultrasonographer time. However important techniques such as the "3D-reverse face" view technique[8] and improved ultrasonography equipment and probes will also lead to better visualization of the palate. Martinez-Ten *et al*[9,23] described the importance of incorporating the "reverse-face view, with the flipped-face views and subsequently the oblique-face view into the algorithm of analysis a possible CP in the neonate. They also demonstrated that an accurate visualization of the palate required good initially acquired volume, fluid between the fetal tongue and palate, and curving of the plane to follow the structure of the palate[23]. Subsequently, the oblique-face or flipped-face view makes better visualization in selected cases.

CLASSIFICATION

Orofacial clefting is multifactorial and etiologically heterogeneous. Therefore, proper classification is essential as different types of clefts may be variably associated with additional anomalies and chromosomal disorders. Over the years many different classification systems based on morphological, anatomical or etiological features of OFCs have been proposed. However, postnatal classification may not be applicable to prenatal findings.

An antenatal sonographic classification system has been proposed by Nyberg *et al*[25,26]. This classification

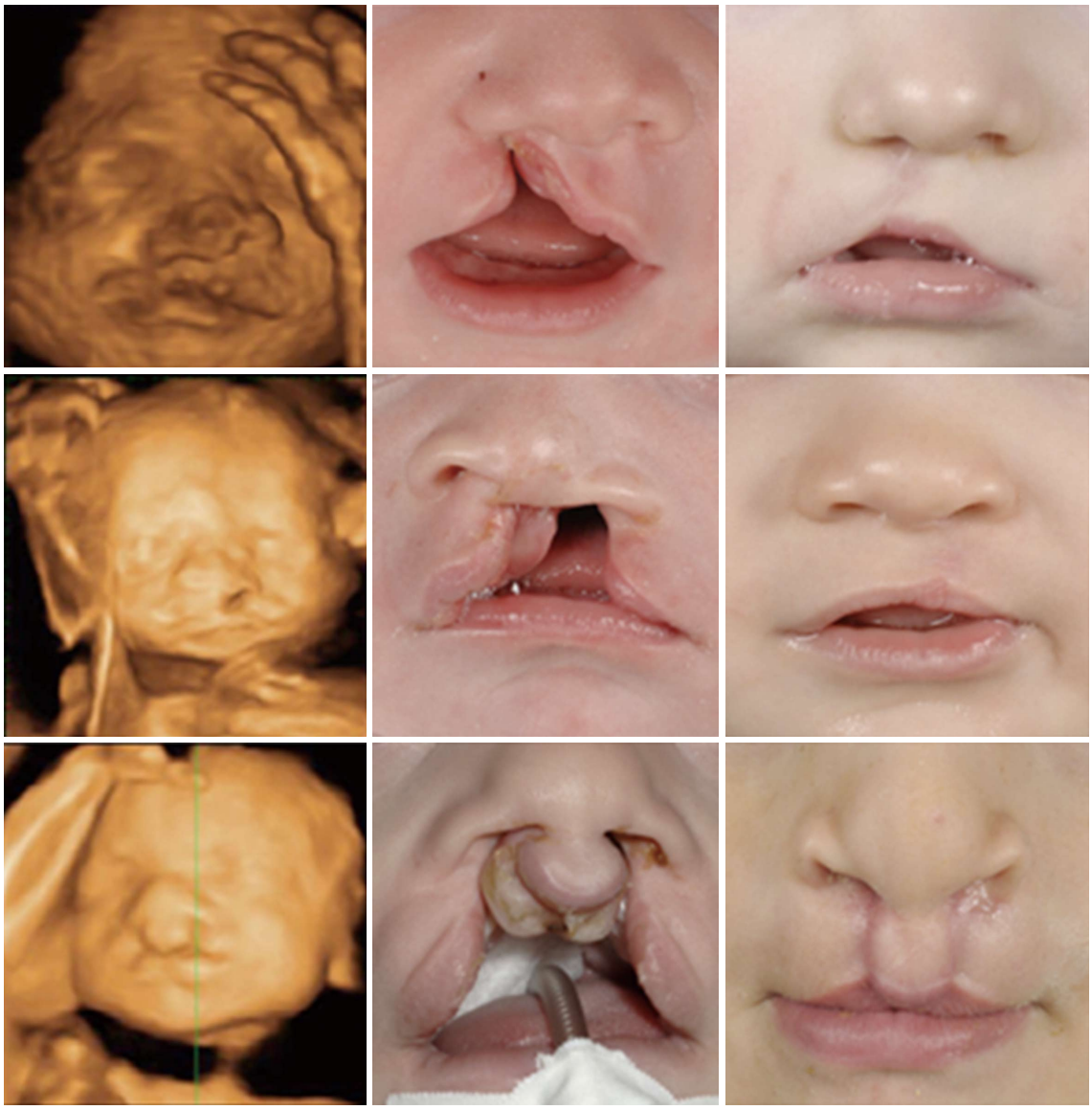

Figure 4 Prenatal, postnatal and post-surgery images of three different patients with cleft lip and alveolus, cleft lip and palate and bilateral cleft lip and palate respectively.

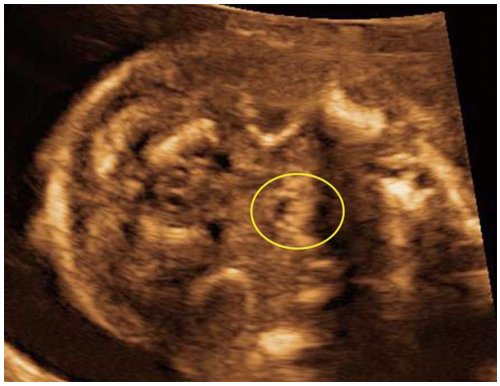

Figure 5 Echo pattern of a normal uvula visualized by ultrasound is typical and strongly resembles an "equals sign".

shows four types of clefts and their relationship with the primary and secondary palate. Type 1: Isolated CL alone, type 2: Unilateral CL and palate, type 3: Bilateral CL and palate and type 4: Median CL and palate. The so called type 5 clefts is another group of facial clefts associated with the amniotic band syndrome or the limb-body-wall complex and does not follow embryologic patterns but rather shows random types of often large and devastating defects[25]. They further suggest that type 1 clefts are associated with a low rate of anomalies and type 2 and 3 clefts with intermediate prognosis. Type 4 and 5 clefts are universally associated with concurrent anomalies and with fatal outcome[26]. The Nyberg classification has several shortcomings; CP for instance is not mentioned at all, while not all midline clefts have a fatal outcome.

A myriad of classification systems has been proposed and utilized over the years, however only a few have found clinical application. The most generally accepted classification was developed by Kernahan in

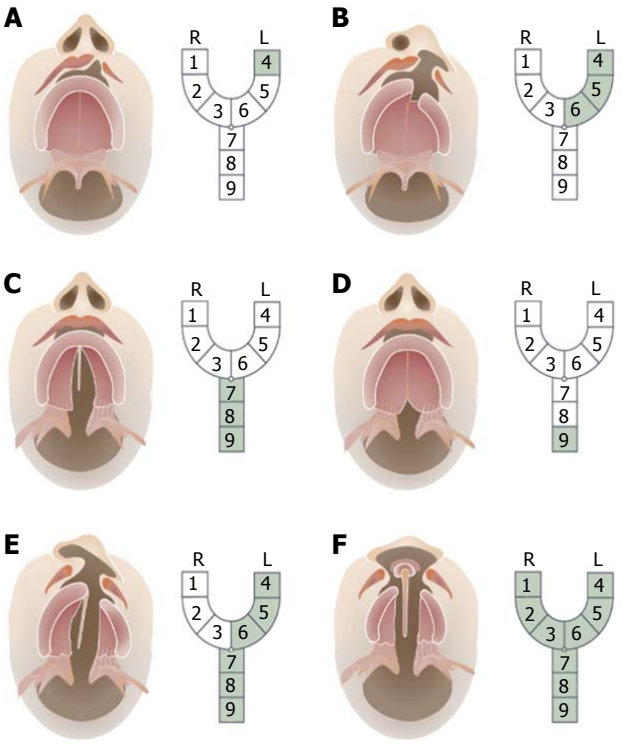

Figure 6 Kernahan's classification. The area affected by the cleft is labeled from 1-9, each of which represents a different anatomical structure: 1: Right lip; 2: Right alveolus; 3: Right premaxilla; 4: Left lip; 5: Left alveolus; 6: Left premaxilla; 7: Hard palate; 8: Soft palate; 9: Submucous cleft.

1971 (Figure 6), who proposed a striped Y-classification with the incisive foramen as the reference[27]. This system is based on the resemblance of an intra-oral view of a CL and palate to the letter "Y". The area affected by the cleft is labeled from 1-9, each of which represents a different anatomical structure.

There has been a general move to adopt a simple classification system for clefts. The LAHSHAL code is often used as the preferred classification system by cleft surgeons ("L" = lip, "A" alveolus, "H" hard palate, "Soft palate"). It is compatible with ICD10 and allows clefts to be coded for computer use. The LAHSHAL codes split the relevant parts of the mouth in six parts and is written from the perspective of someone looking at the patient (*i.e.*, the first letter is for the patients right lip and the last letter for the patients left lip). The LAHSAL code indicates for each patient whether there is a complete cleft (upper case letter, *e.g.*, "L") or an incomplete cleft (lower case, *e.g.*, "l") or no cleft. LAs would subsequently connote a complete cleft of the right lip/alveolus and incomplete cleft of the soft palate. SHAL would connote a complete left-sided unilateral CL, alveolus and palate. The midline Tessier clefts occur very infrequently, and are not included in these classifications.

CONCLUSION

Knowledge of the embryology of the face should add to the understanding and correctly diagnosing OFCs. Failure of fusion between any of the facial swellings results in facial clefts and can occur either unilaterally or bilaterally and typically happens at the junction of the lateral incisor and the first premolar teeth. The depth of the cleft may vary from the soft tissue of the lip to a complete cleft of the maxillary bone. Lateralization of the alar base could help the ultrasonographer to diagnose a complete CLP patient where the visualisation of the CP is difficult. Maxillary protrusion seen with a bilateral CL is highly suggestive of a bilateral complete CL/palate. The embryological fusion of the palatal shelves starts anteriorly and proceeds posteriorly like a zip. An unremarkable uvula, visualised by ultrasound as an "equals sign", suggests an intact normal palate. If the ultrasonographer cannot visualise the middle part of the secondary palate, but the "equals sign" is visible, it is suggestive of an intact palate. This could safe the ultrasonographer time.

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P- Reviewer: Mattos BSC **S- Editor**: Ji FF **L- Editor**: A
E- Editor: Li D

World Journal of Methodology

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

World J Methodol 2017 September 26; 7(3): 101-107

DOI: 10.5662/wjm.v7.i3.101

ISSN 2222-0682 (online)

MINIREVIEWS

Pancreatic imaging: Current status of clinical practices and small animal studies

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Author contributions: Ni Y set up the format and the frame of this mini-review article, and final approval of the manuscript; Yin T wrote and edited the manuscript; Peeters R approved sections related to MRI techniques and edited the manuscript; Liu Y and Feng Y provided suggestions regarding to clinical aspects.

Conflict-of-interest statement: The authors declare no potential conflicts of interest.

Manuscript source: Unsolicited manuscript

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Received: July 14, 2017
Peer-review started: July 17, 2017
First decision: August 4, 2017
Revised: August 22, 2017
Accepted: September 3, 2017
Article in press: September 4, 2017
Published online: September 26, 2017

Abstract

Different causative factors acting on the pancreas can result in diseases such as pancreatitis, diabetes and pancreatic tumors. The high incidence and mortality of pancreatic diseases have placed diagnostic imaging in a crucial position in daily clinical practice. In this mini-review article different pancreatic imaging techniques are discussed, from the standard clinical imaging modalities and state of the art clinical magnetic resonance imaging techniques to current situations in pre-clinical pancreatic imaging studies. In particular, the challenges of pre-clinical rodent pancreatic imaging are addressed, with both the image acquisition techniques and the post-processing methods for rodent pancreatic imaging elaborated.

Key words: Pancreatic imaging; Rats; State of the art clinical magnetic resonance imaging; 3.0T scanner; Quantitative magnetic resonance imaging

Core tip: In this minireview, the challenges of pre-clinical rodent pancreatic imaging are addressed, basic clinical magnetic resonance imaging techniques and post-processing methods for rodent pancreatic imaging are also elaborated.

Yin T, Liu Y, Peeters R, Feng Y, Ni Y. Pancreatic imaging: Current status of clinical practices and small animal studies. *World J Methodol* 2017; 7(3): 101-107 Available from: URL: http://www.wjgnet.com/2222-0682/full/v7/i3/101.htm DOI: http://dx.doi.org/10.5662/wjm.v7.i3.101

INTRODUCTION

The pancreas is an important visceral organ performing both endocrine and exocrine functions. Abnormalities

of the pancreas result in diseases such as pancreatitis, diabetes, and pancreatic tumors[1,2]. The onset of diabetes is usually long after beta cell dysfunction and insulin resistance[3,4]; pancreatic cancer is generally asymptomatic and frequently diagnosed at a late stage[5]; acute pancreatitis is a painful inflammatory condition often with severe complications and high mortality despite treatment[6], while chronic pancreatitis can mimic the symptoms of pancreatic cancer and lead to misdiagnosis[7]. The high incidence of pancreatitis and diabetes, and poor survival rate of pancreatic cancers have increased the demand for new diagnostic and therapeutic strategies[8,9] Herein multimodality multi-parametric imaging plays an indispensable role in disease detection, therapy guidance and patient follow-up. In this mini-review, current situations of common clinical practices and recent development of pre-clinical rodent studies in pancreatic imaging are inspected and discussed with the emphasis on basic magnetic resonance imaging (MRI) techniques and post-processing methods for rodent pancreatic studies.

OVERVIEW OF CLINICAL IMAGING MODALITIES

Ultrasound

As an initial step, abdominal ultrasound is most commonly used in screening for biliary stones and tumors, as this equipment is widely available at relatively low costs[10]. However, the quality of ultrasound images and diagnostic accuracy are highly user-dependent, and the retroperitoneal location of the pancreas may impose image artifacts and hamper the ultrasound diagnosis[11]. For further confirmation and staging of pancreatic diseases, imaging modalities with higher quality and sensitivity are needed.

Computed tomography

Contrast-enhanced multi-detector computed tomography (MDCT) remains the standard modality in clinic for the assessment of pancreatitis and pancreatic cancer[12,13]. Due to its high spatial resolution and fast image acquisition, MDCT combined with contrast agents injection, has shown its powerful capacity in the staging of pancreatitis and pancreatic cancer with high sensitivity and specificity[7,12].

MRI

MRI as a non-ionizing imaging modality has been increasingly utilized in clinic due to its multi-parametric capability[14]. With the constant improvement of the new MRI hardware and imaging reconstruction algorithms, MRI is currently capable of acquiring images of spatial resolution approaching to that of CT. Meanwhile, with the application of accelerated parallel imaging techniques, most MRI protocols have the feasibility to be accomplished in one or a few breath-holds[14,15].

Traditionally, T2-weighted MRI sequences are commonly used to provide structural information on the anatomy of the pancreatic ductal system and lesions[14]. MR cholangiopancreatography (MRCP) using heavily T2-weighted sequences has been widely applied as non-invasive alternative to endoscopic retrograde cholangiopancreatography (ERCP) for biliopancreatic duct system evaluation[16]. The combination of dual-echo (in/opposed-phase) T1-weighted MRI sequences is useful for hemorrhage and fat content assessment[14]. Dynamic contrast-enhanced (DCE) MRI scans are performed for differential diagnosis and grading of solid pancreatic lesions and pancreatitis by analyzing the pharmacokinetic parameters or contrast concentration curves[17,18]. In addition, diffusion-weighted MRI (DWI) protocols have also shown a great potential to depict and characterize pancreatic diseases including acute/chronic pancreatitis and benign or malignant tumors[19] without a need to use contrast agents.

Other more advanced but less popular pancreatic imaging modalities, often with a certain invasiveness or radiation exposure, include endoscopic and contrast enhanced US (EUS and CEUS), positron emission computed tomography and single-photon emission computed tomography incorporated with X-ray based CT (PET/CT and SPECT/CT) for improved spatial resolution and co-localization of imaging findings, *etc*. For a more comprehensive overview, a recent review article about imaging pancreatic diseases is recommended[5].

CURRENT STATUS OF RODENT PANCREATIC IMAGING

Challenges in rodent pancreatic imaging

In order to develop new diagnostic and therapeutic strategies against pancreatic diseases, rodent models are commonly used in preclinical studies. However, imaging the pancreas in rodents proves to be extremely challenging due to motion artifacts and uncertain anatomy. The pancreas in humans represents a retroperitoneal solid organ, which can be identified by imaging modalities even without contrast enhancement, as stated above. However, unlike the human pancreas, the rodent pancreas appears as a soft, diffuse and irregularly lobulated organ, which is very difficult to discern from surrounding tissues[19-22], even during open abdominal surgery (Figure 1). Pancreas-specific contrast agents would facilitate pancreas visualization, but currently those pancreatic specific markers are unavailable yet. Without specific labeling, rodent caudate liver lobes and abdominal fat tissue are frequently identified as pancreas by mistake. In some animal studies, pancreas associated injuries in other solid organs, instead of the pancreas itself, were investigated using contrast-enhanced protocols and MR spectroscopy (MRS)[23,24].

Three dimensional pancreatic imaging

To avoid the misdiagnosis and to have a detailed

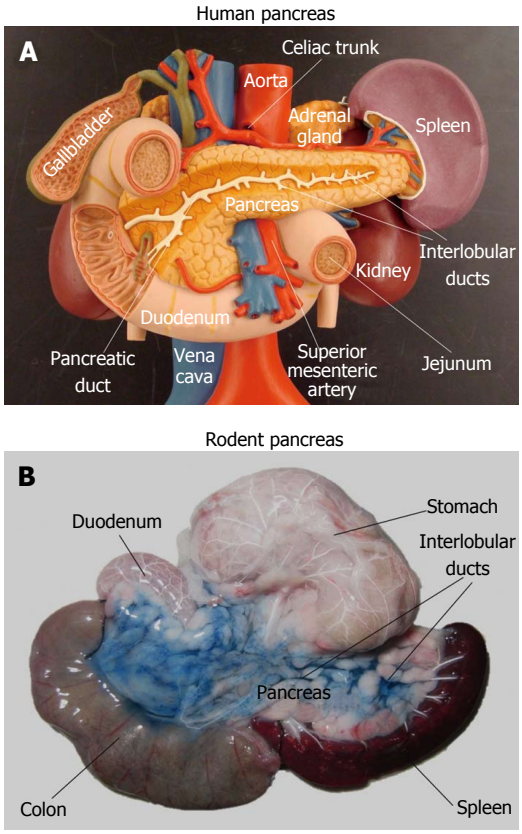

Figure 1 Anatomical difference between human and rodent pancreas. Unlike the human pancreas which is a well-defined solid organ (A), the rat pancreas appears as a soft, diffuse and irregularly lobulated organ (B), which is very difficult to discern from surrounding tissues even at open abdominal surgery. To better visualize it, the pancreatic ductal system was infused with Evans blue dye while the arterial system was injected with a barium sulphate suspension.

overview of the pancreas anatomy, two pancreatic imaging studies were performed using contrast-enhanced high-resolution three dimensional (3D) modalities to provide more precise anatomical information of the rodent pancreas[20,22]. In a micro-CT study[20], the *in vivo* rat pancreatic tail portion could be identified after a two-step contrast injection. Unlike the human pancreas that can be readily depicted by MRI even without using any contrast agent (Figure 2), detailed 3D rodent pancreatic anatomy and surrounding landmarks could only be demonstrated (Figure 2) by biliopancreatic local infusion of mixed contrast media in a post-mortem model[22].

Diabetes imaging

In order to achieve early diagnosis in diabetes, the development of pancreatic specific contrast agents became a hot topic. Among others, some contrast agents were used to target pancreatic beta cells for diabetes related research subjects, for instance, glucagon-like peptide-1 (GLP1) receptor and GLP1 analogues have been frequently studied in rodent diabetes imaging[25,26]. The micro-vascular changes in case of diabetes and pancreatic inflammation were also investigated[27,28].

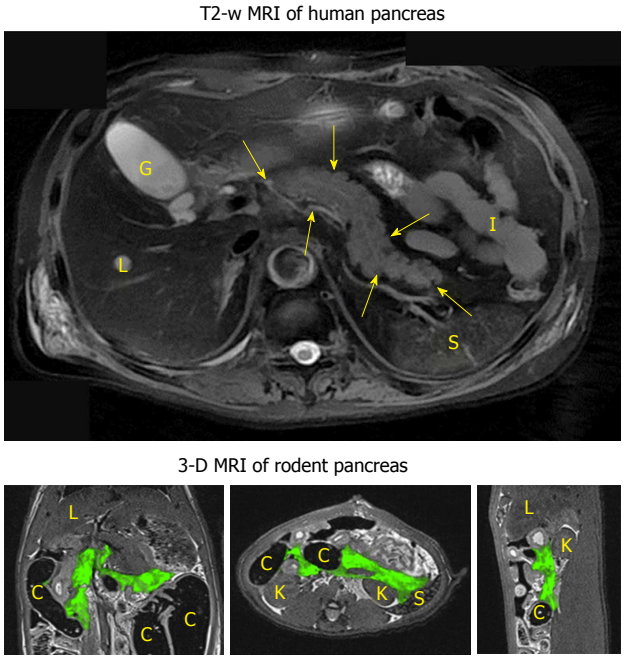

Figure 2 Typical human pancreatic magnetic resonance imaging *vs* rodent pancreatic magnetic resonance imaging. The upper transverse image shows the human pancreas (contoured by arrowheads) as a solid organ adjacent to the liver (L), gallbladder (G), spleen (S), kidney (K) and small intestines (I). The lower 3-D images display the coronal (left), transverse (mid) and sagittal (right) views of the contrast-infused rat pancreas with green color coding, adjacent to the liver (L), spleen (S), kidney (K) and colon (C). MRI: Magnetic resonance imaging.

Pancreatitis imaging

The first attempt of rodent pancreatitis imaging started during the 1980's, conducted by Paajanen *et al*[29], in which Gd-DTPA was applied as a contrast agent for T2/T1 relaxation measurements in an acute hemorrhagic pancreatitis model. In 1989, Kushnir *et al*[30] performed several MRS experiments to identify imaging biomarkers in an acute pancreatitis model. More recently, specific nanoparticles were developed for pancreatitis imaging, with lipase as the target[31]. Imaging of acute edematous pancreatitis can also be performed with MRCP, T2 relaxation measurement and non-specific contrast enhancement using modified protocols on a state of the art clinical MRI scanner[32].

Pancreatic tumor imaging

The direct visualization of rodent pancreas and corresponding pancreatic landmarks could facilitate more precise diagnosis of a pancreatic tumor in the early stages. As a tumor grows to a certain volume, the identification of the solid tumor mass is much easier to perform than imaging other pancreatic disorders. Quantitative T2 and T1 relaxation measurements, DWI parameters and perfusion information can be obtained using multi-parametric MRI[33]. Currently, rodent pancreatic tumor models are increasingly used

to investigate new therapeutic strategies in longitudinal follow-up studies by non-invasive MRI.

RODENT PANCREATIC IMAGING USING A CLINICAL MRI SCANNER

Rationale behind the use of a clinical MRI scanner for rodent pancreatic research

Due to the small size of the rodent pancreas, it is necessary to use high resolution 3D anatomical images for precise pancreas localization. Misdiagnosis could be avoided by carefully tracking the anatomy of the surrounding organs or tissues in 3D mode. Unfortunately, 3D anatomical MRI in the abdominal region is extremely challenging in commonly used high-field pre-clinical scanners, due to their high sensitivity to motion artifacts at high magnetic field and unavoidable long scanning durations. Alternatively, by the combined use of dedicated multi-channel coils and accelerated parallel imaging, clinical MR scanners have shown the feasibility and flexibility for rodent abdominal imaging[22,32,33].

Limits and benefits of clinical scanners

The biggest problem using clinical scanners for small animal studies is the limited gradient strength[34]. Most clinical MRI scanners have a gradient amplitude of 40 mT/m and slew rates up to 200 T/m per second. Although the maximum gradient amplitude in the recent 3T Siemens Prisma scanner has been increased to 80 mT/m, the gradient strengths are still up to 10 times lower than that of the current state of the art pre-clinical scanners. Consequently, the minimum slice thickness and minimal field of view (FOV) in the clinical systems are more restricted. In our studies, to maintain enough signal-to-noise-ratio (SNR), most 2D scans were performed with a slice thickness of 2 mm, which is identical to those acquired from small animal scanners. However, the minimal FOV is usually limited to around 70 mm. The limited gradient strengths also hamper the use of echo planar imaging (EPI) due to the prolonged readout, which leads to severe image distortions.

On the other hand, current state of the art clinical MRI scanners provide an excellent hardware stability, higher field homogeneity and a dedicated user interface, and are more widely accessible compared to small animal scanners. With the combination of dedicated clinical multi-channel surface coils and the self-calibrated parallel imaging techniques GRAPPA (GeneRalized Autocalibrating Partial Parallel Acquisition), it is possible to acquire high SNR images in rodent heterogeneous abdominal region with a sufficiently short scan duration. Moreover, lower magnetic field and application of GRAPPA provide a higher feasibility to rodent abdominal imaging.

Basic clinical MRI techniques for rodent pancreatic imaging

In our serial studies[22,32,33], clinical scanners were used for pancreatic imaging: The Magnetom Tim Trio (Siemens, Erlangen, 45 mT/m, 200 T/m per second) combined with an 8-channel wrist coil; and the Magnetom Prisma (Siemens, Erlangen, 80 mT/m, 200 T/m per second) together with a 16-channel wrist coil. There are several standard clinical protocols which can be directly translated to pre-clinical research, including T2-weighted 3D turbo spin-echo (TSE) based SPACE (3D TSE with variable-flip-angle refocusing RF pulses) imaging and MRCP protocols, standard 2D multi-echo spin-echo sequences for T2 relaxation, as well as diffusion and perfusion sequences. The animal models introduced in these studies[22,32,33] are a rat acute pancreatitis model and a rat orthotopic pancreatic tumor model, in which we intended to characterize pathological changes including edema, hemorrhage and necrosis by those modified MRI methods.

Three-dimensional volumetric images: As mentioned above, 3D imaging would facilitate the localization of rodent pancreas. The other benefit is the possibility of volumetric measurements in 3D. In case of edematous pancreatitis, edema volume is a biomarker for pancreatitis. Meanwhile, 3D views could also provide a more accurate measurement for irregularly shaped abdominal tumors (Figure 2). The volume of the target tissue can be obtained from post-process image segmentation. The most important 3D imaging protocols used here are T2-weighted SPACE and MRCP, which are also widely used in clinical MRI abdominal examinations.

Quantitative MRI measurements: Quantitative MRI relaxation measurements are useful in organ/tissue characterization. T2 mapping is helpful in the assessment of fluid content and hemorrhage; and native T1 mapping is essential for the calculation of the tissue concentration time curve (CTC) in DCE protocols. In these studies, mono-exponential T2 mapping and inversion recovery based T1 mapping were performed.

Measurements of Gaussian and non-Gaussian diffusion for water in biological tissues can be accomplished using DWI with different combinations of diffusion weightings. Mean diffusivity and diffusion kurtosis were obtained from 3-trace diffusion images.

Moreover, tissue perfusion can be characterized using DCE protocols, after the injection of a gadolinium based MRI contrast agent. After the signal conversion to gadolinium concentration using pre-contrast native T1 relaxation information, either semi-quantitative information or quantitative parameters from the pharmacokinetic Tofts model were extracted. Detailed MRI protocol parameters are elaborated in the different serial studies[22,32,33].

Data processing

In these studies, open-source software and in-house built programs were used for data processing. This includes image segmentation, registration and 3D image visualization in open-source software ITK-SNAP (www.itksnap.org) and MeVisLab (MeVis Medical Solutions, Bremen, Germany); DICOM process, MRI mathematical modeling and quantitative analysis in Matlab programs (MathWorks, Natick, Massachusetts); and statistical analysis and data visualization using programs combining both Matlab and R (https://cran.r-project.org). Detailed image processing equations are included in the next section.

SOME OF THE ONGOING RESEARCH IN RODENT PANCREATIC MRI STUDIES

Identified objectives

Present research project aims at providing practical solutions to rodent pancreatic imaging using clinical facilities, from *ex vivo* to noninvasive *in vivo* imaging with the following systematic objectives identified: (1) To overcome the limitations of clinical MRI scanner for small rodent imaging studies; (2) detailed visualization of a complete pancreas and topographic landmarks through contrast enhanced CT and MR imaging in a rat postmortem model; (3) to explore noninvasive MR imaging methods for characterization of the Caerulein induced acute pancreatitis in rats; (4) to estimate the reliability of 3D isotropic MRI and quantitative multi-parametric MRI for characterization of an orthotopic pancreatic head tumor model in rats; and (5) to investigate therapeutic response of a vascular disruption agent in rat pancreatic tumor models with further modified quantitative multi-parametric methods.

Processing for quantitative parameters in rodent pancreatic MRI

MRI quantitative parameters can be obtained from advanced image processing methods using machine learning algorithms[35-37]. For practical considerations, quantitative parameters are re-generated from in-house built Matlab programs using non-linear least square methods with CPU (Central Processing Unit) acceleration.

T2 mapping: Traditionally, the transverse relaxation time T2 is obtained using multi-echo spin-echo pulse sequences, by sampling signals at several different echo-times (TE), and fitted to either multi- or single-exponential decay functions[38]. Fast T2 mapping can be obtained using balanced steady-state free precession (SSFP) readout[39].

T1 mapping: On a clinical scanner, fast T1 mapping can be measured using inversion recovery methods or from variable flip angles experiments[39,40]. Since the MRI acquisition has to be synchronized with animal respiration, the effective repetition time (TR) is usually longer than 1 s. Thus, inversion recovery based protocols would be suggested for T1 mapping in rodent pancreatic imaging. Typically, the equation for measured signal in the inversion recovery T1 mapping experiment is a three-parameter function: $SI_{(t)} = a + b \times \exp(-t/T1^{*})$, where $SI_{(t)}$ is the signal intensity after each inversion time t, and $T1^{*}$ is the effective longitudinal relaxation time. The actual T1 relaxation time can be obtained after correction for the flip angles[41], or the Look-Locker readout[42].

Diffusion-weighted model: In DWI experiments, the simple Gaussian diffusion can be assumed using a mono-exponential model. The two-compartment intravoxel incoherent motion model on the other hand is currently widely used in clinical pancreatitis and pancreatic tumor studies[43,44], and separates diffusion into the true-diffusion and the pseudo-diffusion fraction. Alternatively, sampling with high b-values above 1000 s/mm^2 can be applied for non-Gaussian diffusion estimation using a diffusion kurtosis model[45].

Post-processing for DCE model: The first step in DCE data post-processing is the conversion of the raw MRI signal to the tissue concentration time curve (CTC). The tissue concentration C_t of contrast agent (CA) during the DCE perfusion experiment is solved as: $1/T1_{(t)} = 1/T1_0 + r1 \times C_{t(t)}$, where $T1_0$ is the T1 value before contrast injection, obtained from inversion recovery T1 mapping, and r1 is the longitudinal relativity of the applied CA. In a high temporal resolution DCE experiment, the T1 relaxation $T1_{(t)}$ after CA injection can be converted from the signal intensity (SI) time curve as described previously[33]. Alternatively, CTC information can be directly extracted from the dynamic T1 mapping.

The vascular input function (VIF) C_p is determined by the CA concentration in blood C_b: $C_p = C_b/(1 - Hct)$, which is obtained from CTC of the aorta or a major vein, and the hematocrit level Hct which is set to 42% in our studies. VIF is usually fitted into a bi-exponential function for further kinetic modeling. Perfusion indices, the transfer coefficient *Ktrans* and the rate constant *kep*, can be obtained from the standard or the modified Tofts model[46]. In practice, the discrete convolution can be constructed as a matrix multiplication. The fraction volume *ve* of extravascular extracellular space is calculated as: ve = Ktrans/kep.

CONCLUSION

The diagnosis of pancreatic diseases and their management have been largely facilitated by ever advancing multimodal and multi-parametric imaging technologies in clinical settings. Likewise, thanks to the above-mentioned efforts, preclinical research on rodent models of pancreatic pathologies are also rapidly progressing in terms of visual identification

of rodent pancreas on 2D and 3D images, imaging characterization of common pancreatic disorders such as pancreatitis and pancreatic malignancies, and noninvasive imaging follow-up of investigative therapies for new drug development. Eventually clinical practices in patients suffering from those often deadly diseases on this complex visceral organ of pancreas will benefit from all these translational studies.

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P- Reviewer: Barauskas G, Weng CF **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Li D

World Journal of Methodology

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

World J Methodol 2017 September 26; 7(3): 108-111

DOI: 10.5662/wjm.v7.i3.108

ISSN 2222-0682 (online)

ORIGINAL ARTICLE

Retrospective Study

Accuracy of crescent sign on ocular ultrasound in diagnosing papilledema

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Author contributions: All authors equally contributed to this paper with conception and design of the study, literature review and analysis, drafting and critical revision and editing, and final approval of the final version.

Institutional review board statement: This study was reviewed and approved by the Institutional Review Board of Aravind Eye Hospital.

Informed consent statement: Patients were not required to give informed consent tothe study because the analysis used anonymous clinical data that were obtained after each patient agreed to treatment by written consent.

Conflict-of-interest statement: The authors have no financial relationships to disclose.

Data sharing statement: No additional data are available.

Manuscript source: Invited manuscript

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Received: January 27, 2017
Peer-review started: February 8, 2017
First decision: June 12, 2017
Revised: July 22, 2017
Accepted: August 2, 2017
Article in press: August 2, 2017
Published online: September 26, 2017

Abstract

AIM

To study the usefulness of orbital ultrasonography in the diagnosis of papilledema.

METHODS

Fifty patients who were referred to the neuroophthalmology clinic and clinically suspected to have papilledema were selected. Thorough, clinical examination with slitlamp biomicroscopy and visual acuity assessment was done. These patients underwent ultrasonography to demonstrate the crescent sign. The patients were further evaluated with the neurologist and magnetic resonance imaging (MRI) thus confirming the diagnosis of papilledema. The results of our ultrasonographic evaluation were correlated with final diagnosis after thorough clinical evaluation, imaging and the neurologist's opinion.

RESULTS

Out of 50 patients diagnosed having papilledema on MRI, 46 (92%) showed crescent sign on B scan ultrasonography. Headache was most common presenting complaint in 47 (94%) and idiopathic intracranial hypertension was most common underlying cause of papilledema in 30 (60%) cases.

CONCLUSION

"Crescent sign" seen on ultrasonography is a sensitive tool for diagnosis of papilledema. It is cost effective, less cumbersome and effective tool to differentiate

between papilledema and pseudo papilledema before subjecting the patients to costly investigations like MRI. A positive crescent sign should always be followed by MRI to find out the cause of papilledema.

Key words: Papilledema; Orbital ultrasound; Doughnut sign; Crescent sign; B scan

Core tip: It is a retrospective study of 50 clinically diagnosed cases of papilledema where 46 cases showed a positive crescent sign on ocular ultrasonography. With an accuracy of 92%, ocular ultrasonography could be a cheaper and useful tool to confirm papilledema before subjecting these patients for MRI scans.

Bhosale A, Shah VM, Shah PK. Accuracy of crescent sign on ocular ultrasound in diagnosing papilledema. *World J Methodol* 2017; 7(3): 108-111 Available from: URL: http://www.wjgnet.com/2222-0682/full/v7/i3/108.htm DOI: http://dx.doi.org/10.5662/wjm.v7.i3.108

INTRODUCTION

Patients of papilledema have acquired bilateral, optic nerve head swelling due to increased intracranial pressure[1]. Papilledema is an important reason of referral of patients to neuro-ophthalmology clinic. Suspicion of papilledema warrants timely ascertaining of its diagnosis, evaluation and management[2]. Differentiation between papilledema and pseudopapilledema is important as patients with papilledema need thorough work up whereas patients with pseudopapilledema often only need reassurance monitoring and follow-up. The presentation of papilledema patients is varied, some present with headache, transient visual blurring, *etc.* Pseudopapilledema might present with more severe and acute visual presentation. To differentiate these and confirmation of diagnosis is usually done by magnetic resonance imaging (MRI). MRI is a tedious, costly investigation. Ultrasonography is a safe, cost-effective, easily available, non-cumbersome modality for diagnosis of papilledema. There are very few studies done which demonstrate the value of ocular ultrasonography in diagnosis of papilledema. Some studies have shown increased optic nerve sheath width by orbital ultrasonography correlating well with the final diagnosis of papilledema or pseudo papilledema[3-5]. However all these studies measured the optic nerve width ranging anywhere from 3 mm to 3.3 mm to 5.7 mm along with 30° test. Measuring the optic nerve width and 30° test is difficult and requires an experienced ultrasonographer. None of the studies emphasized on the presence of "crescent" or "doughnut" sign alone, which is easier to elicit and does not require a very experienced or skilled ultrasonographer. Our study shows the effectiveness of eliciting the "crescent" sign on ocular ultrasonography in diagnosing papilledema, before subjecting them to costly investigations like MRI.

MATERIALS AND METHODS

This was a retrospective study done in our institute from August 2015 to July 2016. Fifty patients diagnosed to have papilledema clinically, were included in this study. For each patient age, sex, duration of complaints, headache if present, associated systemic conditions were recorded. After measuring the visual acuity, all patients were clinically examined first by torch light, followed by slit lamp examination, intraocular pressure recording by applanation tonometry and a dilated fundus examination using 90 diopter lens. Informed consent was taken from all patients. Ocular ultrasonography using a 10 MHz probe (Sonomed Escalon E-Z Scan B5500+, Wayne, PA, United States) was performed on all in a supine position. If fluid was seen around the optic nerve, within the sheath, then it was noted as "crescent" or "doughnut" sign positive and it indicated presence of papilledema (Figure 1). All patients were referred to a neurologist and all crescent sign positive cases were subjected to neuro imaging. SPSS version 16.0 software was used for the statistical analysis.

RESULTS

The average age of presentation was 30 years (range 6 to 60 years). There were 37 (74%) females and only 13 (26%) males. Headache was the most common presenting complaint seen in 47/50 (94%) cases. Transient visual loss and diplopia were other complaints in very few cases. The duration of symptoms ranged from four days to 4 years. The most common systemic association was hypertension and diabetes mellitus in 3 patients (6%) each. Seventy-six percent (n = 38) patients out of 50 had no systemic disease.

Out of the 50 diagnosed cases of papilledema 46 showed positive "crescent sign" on ultrasonography. Thus the sensitivity of orbital ultrasonography in diagnosing papilledema was 92% (n = 46). Neuro imaging was done in all these cases and the most common cause of papilledema on MRI was idiopathic intracranial hypertension (IIHT) in 30 cases (60%) followed by space occupying lesions (SOL) in 8 (16%). Of the SOL cases, four had tumours in posterior cranial fossa, one in frontal lobe, two had meningiomas and one had craniopharyngioma. Sinus thrombosis was seen in 9 cases (18%). The common sinuses involved were sagittal sinus, sigmoid sinus and transverse sinus. Two patients had Grade Ⅳ hypertension retinopathy associated papilledema while one patient had multilocular hydrocephalus post shunt surgery. All these three cases along with one case of IIHT had normal

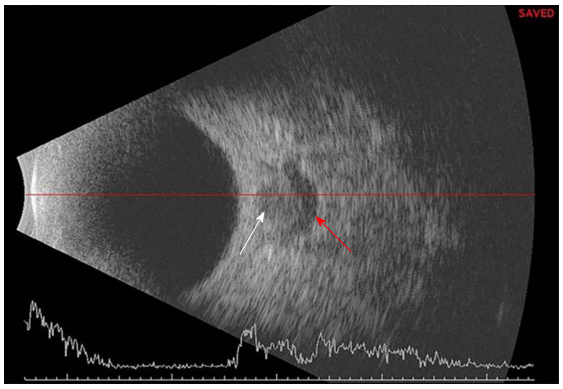

Figure 1 B scan ultrasound picture showing the normal round hypoechoic area of optic nerve (white arrow) with a hypoechoic crescent shaped area (red arrow) depicting "crescent" or "doughnut" sign.

ultrasonography (false negatives).

DISCUSSION

Papilledema is an important reason for emergency referral for patient to neurophthalmology clinic[6]. However, sometimes it is difficult to differentiate papilledema from pseudo papilledema and patient has to be subjected MRI for confirmation of diagnosis[7]. Cost, time duration, difficulties in claustrophobic and metal implants patients make it a tedious investigation. Ocular ultrasonographic measurement of optic disc width along with 30° test and presence of fluid around the optic nerve as a "crescent" or "doughnut" sign has been shown to be useful in confirming papilledema. However these tests, especially measurement of optic disc width and 30° test, require a cooperative patient with an experienced ultrasonographer and these tests are also time consuming. Eliciting the crescent sign alone is faster, has a shorter learning curve and can be done even by a less experienced ultrasonographer. In our study crescent sign was seen in 46/50 cases with a 92% sensitivity, which proves ultrasound B scan is a sensitive tool in initial diagnosis of papilledema. Sensitivity was 90%, 95% and 100% in studies by Neudorfer *et al*[3], Carter *et al*[4] and Mehrpour *et al*[5] respectively. However all these studies took a combination of measuring optic nerve width, 30° test and presence of crescent sign. Our study shows that presence of crescent sign alone has a good sensitivity of 92%. The advantage of eliciting the crescent sign is that it is faster and easier to perform compared to the other two tests. Four cases in our study had false negative scans. Of these two had grade IV hypertensive retinopathy (where we don't expect to have positive crescent sign), one case was with IIHT and another one had hydrocephalus post shunt surgery. In a single case by Sadda *et al*[8] the patient had bilateral disc edema with positive crescent sign on ocular ultrasonography but the patient was symptomless with normal intracranial pressure and normal MRI. None of our cases showed false positivity.

Other investigative modalities like optical coherence tomography (OCT) and fluorescein angiography have also been studied in the diagnosis of papilledema[9,10]. However, retro orbital anatomy cannot be imaged by OCT and fluorescein angiography has disadvantage of being invasive.

In conclusion, our study demonstrates, "crescent sign" on ultrasound for diagnosis of papilledema a highly sensitive tool in differentiating papilledema from pseudo papilledema before subjecting the patients to more costly, tedious and time consuming investigative modalities like MRI. A positive crescent sign should always be followed by MRI to find out the cause of papilledema.

COMMENTS

Background

Papilledema is a critical ocular sign which could be devastating and can lead to permanent disability. Confirmation of diagnosis is usually done by magnetic resonance imaging (MRI), which is a tedious, costly investigation.

Research frontiers

Ocular ultrasonography is a cost-effective, easily available, non-cumbersome, safe modality for diagnosis of papilledema.

Innovations and breakthroughs

There are very few studies done which demonstrate the value of ocular ultrasonography in diagnosis of papilledema. However all these studies measured the optic nerve width along with 30° test. Measuring the optic nerve width and 30° test is difficult and requires an experienced ultrasonographer. None of the studies emphasized on the presence of "crescent" or "doughnut" sign alone, which is easier to elicit and does not require a very experienced or skilled ultrasonographer. This study shows the effectiveness of eliciting the "crescent" sign on ocular ultrasonography in diagnosing papilledema.

Applications

The study results suggest that "crescent sign" seen on ocular ultrasonography is a sensitive and cost effective tool for diagnosis of papilledema.

Terminology

Papilledema is a serious condition of the eye which can have devastating complications. The infective organisms reach the ocular tissues *via* the blood stream. Ocular ultrasonography is readily available instrument with ophthalmologists. It is less cumbersome and can be quickly performed.

Peer-review

This study has valuable data that would be of interest if published.

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P- Reviewer: Nowak MS **S- Editor**: Kong JX **L- Editor**: A
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