Author's response to reviews

Title: Prevalence of low glomerular filtration rate, proteinuria and associated risk factors in North India using Cockcroft-Gault and Modification of Diet in Renal Disease equation: an observational, cross-sectional study.

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Author's response to reviews: see over
To Melissa Norton, MD
Editor-in-Chief
BMC Nephrology

Subject: Resubmission of research article manuscript: Prevalence of low glomerular filtration rate, proteinuria and associated risk factors in North India using Cockcroft-Gault and Modification of Diet in Renal Disease equation: an observational, cross-sectional study

Dear Dr. Norton

Thank you for the review of our manuscript. We appreciate the comments of the reviewers and have taken a great care to address their concerns. We have attached a revised manuscript incorporating the suggestions of the reviewers. Here is our response to reviewer comments.

Reviewer #1

Major points

1) The CG equation was originally made for estimation of creatinine clearance in White population. I am wondering whether the application of CG equation for Indian population to estimate GFR is appropriate. Generally, the eGFR estimated by CG is higher than the eGFR estimated by MDRD equation because it could estimate creatinine clearance. In contrast, MDRD equation may overestimate GFR if the authors did not use the Indian coefficient. This issue should be discussed by citing a manuscript written by Imai et al Am J Kid Dis Dec;50(6):927-37, 2007.

Response: We agree with the concern over application of CG and MDRD equation in Indian population. We acknowledge that a reliable estimate of CKD or low eGFR prevalence is not feasible till GFR estimating equations validated for indigenous population are derived. Whether these equations are then appropriate for estimating GFR in Indian population is subject to appraisal. In this context a study by Srinivas et al, cited in the manuscript, suggests that of all the available serum creatinine based GFR estimating equations CG GFR and MDRD2 equation fare the best in terms of bias, precision, accuracy and correlation in healthy adult Indian subjects. They have also suggested a novel GFR estimating equation for Indian population but it needs validation. Use of a coefficient (in MDR equation) validated for local population is indeed critical before an accurate assessment of GFR can be made. However, this coefficient has not been derived/validated for Indian population. The manuscript by Imai et al reaffirms the significance of this issue and has been incorporated as a citation in the revised manuscript.

2) If the serum creatinine was not calibrated, the value of eGFR>60ml/min/1.73m² are
not reliable. The authors should not show the data of CKD stage 1 and stage 2.

Response: Prevalence data on eGFR >60ml/min/1.73m$^2$ has been removed from the revised manuscript.

3) For variables with normal distribution, $t$-test rather than Wilcoxon's rank sum test is more appropriate.

Response: We agree that $t$-test is the uniformly most powerful unbiased test when the data is normally distributed. $t$-test has a small power advantage over Wilcoxon Mann Whitney test (WMW) in normal distributions. However, for non-normal distributions WMW can be 3-4 times more powerful than $t$-test. Of the continuous variables only age, BMI, WC and hemoglobin were normally distributed. For simplicity we had analyzed all continuous variables with WMW even if they were normally distributed for the gain in power would have been very small if the same analysis was carried out by $t$-test. However, we have taken a note of the suggestion and have analyzed the above mentioned normally distributed variables using $t$-test. Tables have been modified to reflect changed markers of central tendency for these variables in the revised manuscript.

4) In table 4, authors did not include possible confounding factors such as smoking habits, job status, and proteinuria though these are significant factors on univariate regression analyses in Table 2 and 3. Table 4 should be corrected with these variables.

Response: The multivariate logistic regression was fitted by sequentially dropping insignificant variables until only statistically significant variables were left. Smoking and job status were rendered insignificant in multivariate regression model and hence excluded in the final model (Table 4). Proteinuria however still remained significant. We have included proteinuria in the final model in the revised manuscript.

Minor points

1) Is it appropriate to express eGFR <60 (CKD stage: 3-5) as low eGFR or renal impairment? Isn't it a bit subjective?

Response: One of the aims of our study was to assess the appropriateness of GFR estimating equations which are based on Caucasian cohorts in Indian population. The mean GFR of a healthy Indian adult is much lower than his/her western counterpart. In fact using K/DOQI guidelines, 37.9-51.8% of general Indian population was staged as possible CKD stage 2 (60-89 ml/min/1.73m$^2$). This further highlights the need of a GFR estimating equation validated for Indian population. We agree that classification of Indian subjects as renal impairment based on K/DOQI guidelines is questionable and a matter of personal opinion.

Note: The prevalence data on eGFR >60ml/min/1.73m$^2$ has been removed from the revised manuscript.

2) Delete vertical lines in all the tables.
Response: Vertical lines deleted in revised manuscript.

3) Units of Hb are wrong: gm/dl must be corrected in Table 2 and 3.

Response: Units corrected to g/dl in revised manuscript.

4) Better to describe concentrations of Hb: 11.0 than 11.

Response: Hb concentrations represented as mean (SD) in revised manuscript.

5) Need not to present the number of patients with serum creatinine >1.8 mg/dl for readability.

Response: Data removed from revised manuscript.

6) Figure 2 should be simplified: X axis for eGFR strata and Y axis for the proportion of proteinuria. Absolute number does not count for much.

Response: The data on proportion of proteinuria in different eGFR strata has been incorporated in Table 1 of the revised manuscript. Figure 2 has been replaced by a figure giving Bland-Altman plots for eGFR estimation by CG and MDRD equations.

7) Discussion is overly long; need to be shortened.

Response: Discussion shortened in the revised manuscript.

8) Generally speaking, data are expressed as the means ± SD, or the median [range or interquartile range] for normal and non-normal continuous variables, respectively, though in this article, variables are expressed as the median with range. For parameters such as BMI, means ± SD would be preferable.

Response: For continuous variables with normal distribution, we have expressed the data as mean ± SD in the revised manuscript.

9) Were there no interactions between variables?

Response: The logistic regression was done adjusting for main effects as well as significant two way interactions between the variables which contributed significantly to the model. For instance, there were interactions between age>60*HTN, age>60*DM, less than primary*gender, proteinuria*HTN. We however did not consider higher order interactions.

10) Does odds ratio of 0 in age group 20-39 in Table 5 stands for reference? If so, "ref" is appropriate.
Response: Odds ratio of 0 stands for reference group. Replaced it with “Referent” in the table 5 in revised manuscript.

Reviewer #2

Major Compulsory Revisions

1) The main concerns is related to the mis-interpretetion of the information obtained by an cross-sectional study. Indeed, this design does not allow revealing cause-effect relationship but only associations. This is the case of the relationship between obesity and the presence of CKD. On this regard, the term predictor must be deleted throughout the manuscript because it is impossible to predict an event (in this case the CKD) by using cross-sectional data. Therefore, also the comparisons made by the Authors with other “prospective” studies on this issue (obesity and CKD) seem inappropriate.

Response: We agree that cross-sectional studies can only assess statistical associations between exposure and predicted event. Causality can be established only by study designs which include a time dimension like prospective cohorts. We acknowledge that the manuscript does give the impression of establishing a causal inference. We have removed the term predictor throughout the manuscript and have also restricted the supporting citations only to studies with a cross-sectional design, thus enabling valid comparisons.

2. The most clear example of this misinterpretation can be found when Authors discuss on the proteinuria result (The presence of proteinuria significantly increased odds of having CKD. Similar opinions were expressed in a study on Japanese volunteers, where detection of proteinuria by dipstick increased risk of having ESRD 14 times [43]). The present study simply shows that proteinuria is associated with low eGFR (cross-sectional design) but not that it is the cause of CKD; therefore, this result cannot be compared to Okinawa study [Ref 43] (observational prospective study).

Response: The misinterpretation in question has been dropped from the revised manuscript. We have also added the limitations of using a cross-sectional design in the discussion of the revised manuscript.

3. The same holds true when examining results on smoking habit and alcohol intake. Once again, no cause-relationship can be achieved by this cross-sectional study!. On the other hand, it seems reasonably to hypothesize that the lower prevalence of smoking and alcohol use be secondary to the awareness to the awareness of having CKD.

Response: We have dropped citations pertaining to longitudinal data and have retained only those with demonstrated associations in a cross-sectional design. The suggested hypothesis was considered for differences in prevalence of smoking and alcohol intake but a vast majority of subjects were unaware of their disease.

4. On this regard, have Authors recorded this information (patient’s awareness of CKD) in the questionnaire like they did for hypertension and diabetes?
Response: We did collect information regarding CKD awareness. Only 3.3% of subjects with low eGFR (by CG formula) were aware of their disease. Females were more likely to have a lower awareness. We have added the data on low eGFR awareness in the results and discussion of revised manuscript.

5. Authors should more extensively explain why the two formulas used gave an estimated prevalence so different and if there are clear data supporting the choice of either MDRD (Ref 6) or Cockcroft-Gault (Ref 7) equation. Authors should consider including a figure reporting the Bland-Altman analysis or a 2-panel figure with frequency of eGFR measurement with both MDRD equation and Cockcroft-Gault formula.

Response: Both MDRD2 and CG-GFR equations correlate better with actual GFR (DTPA estimated) than other predicting equations in Indian subjects. However studies (Ref. 6 and 7 of manuscript) have shown that MDRD2 equation has a positive bias and thus may overestimate the GFR. CG-GFR equation is however least biased. Besides the exact correction factor (in MDRD equation) has not yet been validated for Indian population. Using an incorrect correction factor can overestimate GFR and thus reduce low GFR prevalence. We have incorporated this discussion in the revised manuscript. A figure giving Bland-Altman plots for males and females has been included in the revised manuscript.

6. Authors deliberately do not stage CKD (even though this information at least for stage 3 to 5 is already reported in Table 1). Why? On the contrary, I believe that such information is important in order to compare different national data. Their concern about the single creatinine measurement can be overcome by considering that the vast majority of studies on CKD prevalence are based on single value and that in a screening study at the laboratory level, Garg et al (Garg AX, et al: Identifying individuals with a reduced GFR using ambulatory laboratory database surveillance. J Am Soc Nephrol 16:1433-1439, 2005) showed that the size of this problem is not relevant.

Response: We agree that majority of studies estimating CKD prevalence have used a single measurement of serum creatinine for pragmatic reasons. However, most of these studies have used GFR estimating equations validated for indigenous population; typically MDRD2 equation with modified correction factors. Secondly, majority of these studies have used standardized serum creatinine values. We did not standardize serum creatinine levels in our study and this raises concerns over the accuracy of GFR estimates, especially at higher values. We have dropped the prevalence data on eGFR >60 ml/min/1.73m² in the revised manuscript and now solely focus on the prevalence of low eGFR (Stage 3-5).

7. Figure 1 is not clear. I suggest to include in panel A median eGFR for males and females and in the panel B the prevalence of low GFR and proteinuria.

Response: Figure redrawn incorporating above suggestions in the revised manuscript. The prevalence data on proteinuria across age groups has been removed from figure 1 for
simplicity.

8) Figure 2 is even less comprehensible (detecting an absolute number on a log-scale is quite impossible). Authors should try to convert this figure in a table that can be eventually added to Table 1.

Response: This figure has been dropped from the revised manuscript. We have incorporated a figure giving Bland Altman plots, as suggested in comment 5. Also we have included data giving proportion of proteinuria across low eGFR strata in Table 1.

Minor Essential Revisions

None.

Discretionary Revisions

1) Analysis of patients with serum creatinine >1.8 mg/dL is not relevant being only functional to the comparison with ref 3. However, if Authors believe this information necessary, I suggest reporting it in the result section rather than in the table.

Response: The data on subjects with serum creatinine >1.8 mg/dl has been removed from the revised manuscript.

2) Description of the multivariate logistic regression model suggests that it is run by using a backward method. If so, it should be added to the paragraph of statistical analysis and deleted from footnotes of Table 4.

Response: The method of logistic regression has been incorporated in the statistical analysis section and explanatory footnote has been deleted from Table 4.

3) What is the CKD prevalence across three socioeconomic strata (rural, urban and semi-urban)? Is there any difference?

Response: The low eGFR (by CG formula) prevalence in rural areas was lower (7.5%) than in urban (16.2%) and periurban (15.3%) areas. This is consistent with studies comparing prevalence of non-communicable disease risk factors in urban and rural Indian population


Response: We did compare our prevalence estimates with African, South East Asian, Chinese and Mexican populations. Also the review paper cited (ref. 19 in original manuscript) summarizes the CKD burden data from various Asian populations. However,
suggested citations are interesting and reaffirm our conclusions that CKD represents a public health problem. We have incorporated the above citations in the revised manuscript.

Sincerely
Narinder P. Singh, MD
Vinay K. Saini, MBBS