Author's response to reviews

Title: High Prevalence of Chronic Kidney Disease in Iran: a Large Population-Based Study

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Author's response to reviews: see over
Dear Dr Kalinka,

Thank you very much for your email message of October, 1, 2008, about the outcome of the peer review of our above-mentioned manuscript and the opportunity to revise and resubmit the paper. We have taken each critique and comment very seriously, and herewith submit a revised version in response to the reviewers’ comments.

As instructed, this is a point-by-point response to the reviewers’ comments and a revised paper with the changes highlighted.

I hereby attest that I have full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Thank you for your consideration.

Sincerely yours

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Reviewer 1: Kunitoshi Iseki
Major Compulsory Revisions

Methods:
If they investigated other lifestyle-related factors such as alcohol drinking or exercise habit, please add it on Table 1. Data of height and weight are needed.
Are Iranian people vegetarian?
Are there any reasons not testing for urine?

OUR RESPONSE: We have not investigated other lifestyle-related factors such as alcohol drinking or exercise habits. Alcohol drinking in Iran is prohibited and hence alcohol drinking is much less than western countries. The means (±SD) of height for men and women were 169.7 (±6.8) and 156.5 (±6.1) respectively and those of weight for men and women were 74.3 (±12.7) and 67.3 (±12.4) respectively.

Iranian people are not totally vegetarian but their dietary meat intake is less than western countries.
As mentioned, in the limitations (discussion part), data regarding urinary albumin and protein excretion were not collected; hence the prevalence of stages I and II CKD could not be estimated in this population.

Results:
Table 2. Prevalence of smoking is less among CKD than that of without CKD.
Gender difference in smoking rate may explain this.

OUR RESPONSE: Table 2: We have mentioned exactly this comment in our discussion as follows: “Another unexpected finding in our study was that, in contrast to other studies, nonsmoking was associated with CKD, although in the multivariate analysis it did not remain in the model. This can be explained by our low prevalence of smoking in comparison to the other studies and also our inability to take into account the dose response relationship between smoking and CKD. In addition, over two thirds of CKD cases were females (70.8%), among whom there was no significant difference between smoker and nonsmoker percentages (3% versus 3.6%, P value=0.32); whereas most of smokers were males, they comprised less than one third of CKD cases (29.2%).”

Table 4. What are the factors adjusted for multivariate model? Did they include age, sex, and BMI in the model?

OUR RESPONSE: The factors adjusted for multivariate model are described in statistical methods section as: “A multivariate logistic regression model was used to estimate the odds ratio (OR) of related factors with CKD. Age (years), sex (reference: male), BMI (reference: < 25 kg/m2), abnormal waist circumference, hypertension, dyslipidemia, DM and smoking in dichotomous fashions were considered as independent variables.”

Discussion
Concerning the explanation of non-significant impact of DM on CKD, if the duration of DM is short, eGFR might be higher than normal (hyperfiltration due to
hyperglycemia). Since the cohort is very large, more than 10,000, their assumption may be incorrect.

OUR RESPONSE: According to our analysis, in our study despite a high prevalence of diabetes in our population, in the multivariate regression model it was not significantly associated with CKD. A probable explanation for this might be the recent development of diabetes in our population, i.e. over than 70% of cases were newly diagnosed. This could be caused by the fact that most of our population were young, i.e. the mean age was 42.7 ±14.9 years (median and IQ25-75 41 and 31–54 years, respectively), and also we have analyzed data of participants of 20 years and over, so most of them did not know that they had diabetes until the lab tests of TLGS revealed it.

Please discuss about the socio-economic conditions of the participants.

OUR RESPONSE:
This part added to discussion section:
In the present study, income and educational levels were the most important determinants of socio-economic status of participants; however because of the homogeneity of the geographical location of participants’ residential area, which is a reliable indicator of their equal economic level; we considered their education as the socio-economic criterion. From this aspect 64% of the participants had the educational level of high school diploma and above and the relationship between CKD and under diploma was significant (data not shown).

Ideally, they should create GFR estimation equation based on inulin-clearance, not adjusting serum creatinine to the Cleveland Clinic.

OUR RESPONSE: Yes, that is right but we have given our limitations that we did not calibrate our serum creatinine measurements according to the Cleveland Clinic standard, where the Modification of Diet in Renal Disease (MDRD) eGFR equation was derived; nor did we validate the MDRD eGFR equation in a local population, and this could also cause an overestimation in the prevalence of CKD.

Reviewer 2: Olafur Skuli Indridason

Major
Creatinine is measured by a Jaffe reaction and the results are not standardized to the current standard of IDMS. While I see this as a major problem, it may only require a better description of the method itself and official reference range for this measurements, as well as discussion of the limitation it encompasses. Neither is done in this manuscript but it would help those that read it in estimating the bias in the estimate of prevalence of CKD in the population, as it is certainly is high. If the reference range is higher than one expects (perhaps 70-120 mcmol/l as in some of the Jaffe reaction methods), the MDRD study equation will underestimate the eGFR (as the eGFR is lower if serum creatinine is higher) and overestimate the prevalence of CKD. This needs to be addressed in the
It would be preferable that all serum creatinine measurements in the world were performed using a method standardized to the IDMS (Isotope dilution mass spectrometry) method but we do not have that possibility yet.

**OUR RESPONSE:**
Sera Creatinine has been determined calorimetrically by Jaffe’s reaction (Creatinine Colorimetric Kit, Pars Azmoun, Tehran, Iran). The assay sensitivity was 0.2mg/dl, intra and inter assay coefficient of variation were 2.5% and 1.9%, respectively. In the method Creatinine reacts with picric acid in alkaline solutions to make a Creatinine picrate complex with maximum absorbance in 492 nm. The assay range was 18-1330 µmol (0.2-15 mg/dl). According to the kit insert, ascorbic acid up to 300 mg/dl, hemoglobin up to 500 mg/dl and lipemia up to 2000 mg/dl has no interference in the reaction. Reference intervals according to producer recommendation were: 53-97 µmol (0.6-1.1 mg/dl) and 80-115 µmol (0.9-1.3 mg/dl) in women and men respectively in serum/plasma. With this reference range which is not too high, we do not expect a considerable underestimation of eGFR by the MDRD study equation. However; as mentioned in the discussion, we agree that measured serum creatinine in our study which is not standardized to the Cleveland Clinic, where the Modification of Diet in Renal Disease (MDRD) eGFR equation was derived; and that we did not validate the MDRD eGFR equation in a local population, could cause an overestimation in the prevalence of CKD.

Other, some major.
Few studies (and not the one referenced) have shown an increase in CKD in general, however there has been an explosion of ESRD patients in the world as the reference they use clearly shows. This needs to be clarified.

**OUR RESPONSE:** Agreed and corrected the references in the manuscript. (reference 2 added to the first point of the background)

The definition of diabetes does not include a measurement of a post-glucose load blood glucose as there is no mention of such a measurement in the method section. This needs to be clarified.

**OUR RESPONSE:** Agreed and corrected. We included the definition of OGTT in the methods section, moreover the statistics were redone. According to our new analysis, the prevalence of diabetes was 13.7% (tables corrected); however diabetes was still not a significant associated factor in the multivariate model.

There is a considerable difference in the male:female ratio which is similar to what has been observed in other studies. This needs to be discussed in that context and the possibility of overestimation of CKD by the equations. Also the male:female ratio f ESRD in Iran should be mentioned in this regard as in most societies there are more men than women (55-60/45-40) entering ESRD registries. It would be informative to have this information to look to in this article. This needs to be discussed in the context of ESRD in Iran and male:female
ration in Iran.

OUR RESPONSE:
In this study which is a population-based one, we are mostly talking about the predialysis phase of chronic kidney disease (CKD). Most of our CKD cases exist in the CKD stages 3 (99.2%). However the number of subjects in the 5th stage of CKD is only 2, therefore with this sample size we can not have any inference regarding male: female ratio.

It is of interest that all measures of body size are related to CKD. To my knowledge there is no evidence that overweight persons (controlled for diabetes and high blood pressure) enter dialysis more frequently than the lighter ones (correct me if I am wrong). This also needs to be discussed in the context of ESRD in Iran, are all dialysis patients fat? On the same note, smokers (who generally are lighter and younger than non-smokers) are less likely than non-smokers to have CKD. I wonder if body size (and therefore muscle mass) is therefore the most important predictor of serum creatinine and therefore the eGFR.

OUR RESPONSE: Again we do not have data of ESRD registries and dialysis patients; what we discuss here is about associated factors in CKD patients who are mainly in stage 3 (eGFR 30-60); in this stage body size is a associated factor of CKD, although it could not be a causal relationship because of the cross-sectional type of our study. Considering that overweight and obesity was an associated factor with CKD in our study, it can be expected that CKD is not more prevalent in smokers than non-smokers (as you have mentioned smokers are generally lighter than non-smokers).

Individual sections:
Abstract:
In the discussion there needs to be more discussion of the limitations of the assay and the equation used.

OUR RESPONSE: We have organized our abstract according to the journal style.

Background, page 3:
First paragraph, first sentence. I believe it is difficult to show data for increasing prevalence of CKD but the reference they use certainly shows an increase in the prevalence and incidence of ESRD, reference 11 in the manuscript might serve to indicate an increase in the prevalence of CKD though.
First paragraph, second and third sentences (ref 3 and 4) there is an certain repetition here and I think there should be an condensation here.

OUR RESPONSE: Agreed and corrected. The references corrected and this part from background removed "It has been shown that risk of death in patients with CKD is two to three folds higher in addition to a higher risk of end-stage renal disease (ESRD) and the likelihood of developing cardiovascular disease.”
Page 4.
“........are outcomes of exposure to ....” I do not understand this part, perhaps they want to say that CKD itself is a risk factor for cardiovascular disease but this needs to be clarified.

OUR RESPONSE: This part wants to say that exposure to cardiovascular disease risk factors such as hypertension, dyslipidemia, diabetes and smoking could be a cause of development of CKD, but for preventing confusion we removed this part of the sentence “or the presence of cardiovascular disease”.

Methods.
The authors describe an elaborate selection process based on households in a Teheran district (District 13). We need to know how the population in this district compares to the rest of the nation and then how those participating in the study compare to those who did not with regard to age (the study sample is relatively young), gender (58% are women but in most countries this is close to 50:50 (knowing some of the gender policies in Iran this may be an issue but there also might be a shortage of men)), race (the MDRD equation incorporates African Americans or black race), presence of treated diabetes and possibly height, weight or BMI or smoking

OUR RESPONSE: District 13 is located in the centre of Tehran and the age and sex distribution of its population is representative of the overall population of Tehran. For comparison with the rest of the nation, we adjusted our data with the data from the 1996 national census. However the reason for no response have been investigated and there was no significant difference regarding age and sex between responders and nonresponders. (Relevant reference sited) But we do not have any data on those who did not participate to compare our participants with regards to weight, BMI, presence of treated diabetes, etc. Bytheway in Iran we do not have AfricanAmericans or black race. For the difference between percentage of female and male, in our study female subjects were more than males but there is no specific gender policy in Iran and this difference is not shown in data of 1996 national census.

Page 5
They say that all blood biochemical analyses were performed at the TLGS laboratory on the day of blood collection. Analyses were performed using the Selectra 2 autoanalyser (Vita........ from the Netherlands........) but then they say that plasma glucose was assayed with an other method and cholesterol with ......This needs to be clarified. In particular, the description of the creatinine assay needs to be described in far more details and how the chosen method compares to other standard methods.

OUR RESPONSE: The Selectra 2© autoanalyser is a machine to perform the laboratory tests, but the methods to do the tests with this autoanalyzer are different for each biochemical blod test, as mentioned in this part. Description of the creatinine assay is given in the first response to the comment of this reviewer.
Page 6. no mention is made of subjects taking lipid lowering medications.

OUR RESPONSE: Agreed and corrected the definition. In the methods subjects taking lipid lowering agents were taken as dyslipidemic and included in analysis.

DM was defined by a method not used, i.e, a 2 hour post load serum glucose but in the method section you only describe a fasting sample.

OUR RESPONSE: Agreed and corrected. We have now added statements as per following (in the Methods section):
Then subjects underwent a standardized 75-g oral glucose tolerance test (OGTT). For 2h-OGTT, 75 g glucose was administrated orally and plasma glucose was measured 2 hours later (2h-PG).

Results.
Please consider along with the overall numbers, to report separately serum creatinine and eGFR for men and women.

OUR RESPONSE: This part added to the results: “the mean (±SD) of serum creatinine and eGFR for men were 1.2 (±0.2) mg/dl and 72.9 (±12.2) mL/min/1.73 m$^2$ respectively and those of serum creatinine and eGFR for women were 1.0 (±0.1) mg/dl and 69.0 (±12.2) mL/min/1.73 m$^2$.”

The age adjusted prevalence is far lower than the prevalence in the cohort (15 vs 19%). Yet, you say that the study sample is young. We need to know the difference between the study sample and the general population in Iran (see point 1 under Methods). + your study examines persons > 20 years of age which is lower than most studies.

OUR RESPONSE: As mentioned before, the age and sex distribution of its population is representative of the overall population of Tehran. For comparison with the rest of the nation regarding age, we adjusted our data with the data from the 1996 national census.

Discussion.
The study shows a remarkable high prevalence of CKD in Iran. Yet the discussion fails to address the most likely cause for this observation, i.e. the difference in the creatinine assay used here and in other studies. It actually also fails to point out clearly the fact that these prevalence numbers actually are similar to some other nations (and the possible explanations for this (again the assay issues come in strong as well as possible issues with population differences). Moreover, they claim that the muscle mass of Asians and Iranians is lower, and the ingestion of meat is lower than in other ethnic groups. This should lead to lower serum creatinine levels and therefore lower eGFR. Their argument that this might lead do higher prevalence of CKD therefore does not seem plausible.
OUR RESPONSE: As mentioned before, we admit that creatinine assay and its standardization is a limitation in our study which is given in the discussion (the last paragraph in limitations): “we did not calibrate our serum creatinine measurements to the Cleveland Clinic, where the Modification of Diet in Renal Disease (MDRD) eGFR equation was derived; nor did we validate the MDRD eGFR equation in a local population, and this could cause an overestimation in the prevalence of CKD” And also for the fact that these prevalence numbers actually are similar to some other nations, we have given information in discussion part, second paragraph as follows: “Not too many studies have reported results similar to ours. The prevalence of CKD stages 3 to 5 in the Japanese general population predicted by the MDRD equation modified by a Japanese coefficient was about 20%[13]. The prevalence of CKD stages 3 and 4 in Thailand estimated recently using the simplified MDRD equation was 13.8%, and the overall prevalence of CKD in a study in Pakistan with a small sample size was 29.9%.” However for the muscle mass point we agree, and removed this part of the discussion from the manuscript: “Second, Asians and probably Iranians generally have lower muscle mass for body weight and lower dietary meat intake than Americans or Europeans; considering the nonenzymatic production of creatinine in skeletal muscle, the amount of creatinine production and, therefore, serum creatinine would be directly related to muscle mass[35], hence it is likely that the lower GFR observed in our study reflects differences in muscle mass and dietary meat intake.” This point could have caused an underestimation of CKD.

The analysis of factors associated with the presence of CKD is interesting an mostly because any measure of body size, age or gender are significant, even though some of these variables are incorporated in the MDRD equation (although not any measure of body size). I do think that this deserves some more discussion of the problems with the equations. While I do not have statistics for Iran, in most western countries, men outnumber women in ESRD. Men also are more likely to develop cardiovascular disease. The males:female issue in CKD therefore does not make sense and has not been addressed adequately in any paper that I know of. Indeed, I suggest the authors redo their statistics for the genders separately.

OUR RESPONSE: As stated in the limitations, MDRD equation is not validated in our local population, and this can cause an overestimation in CKD prevalence. Our purpose was to show the associated factors of CKD in general, however in our study the number of female subjects was more than male subjects and also because of the cultural matters and lower physical activity in females, clustering of cardio metabolic risk factors in Iranian females is more prevalent. Again we insist that we are not talking about ESRD subjects, and we are mostly showing prevalence of CKD and its associated factors in a population whose CKD cases are mostly in stage 3.