PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	A Population-based Comparison of Chronic Kidney Disease Prevalence and Risk Factors among Adults living in the Punjab, Northern India and the United States (2013-2015)
AUTHORS	Bragg-Gresham, Jennifer; Thakur, JS; Jeet, Gursimer; Jain, Sanjay; Pal, Arnab; Prasad, Rajendra; Pennathur, Subramaniam; Saran, Rajiv

VERSION 1 – REVIEW

REVIEWER	Eranga Wijewickrama Faculty of Medicine, University of Colombo, Sri Lanka
REVIEW RETURNED	26-Jun-2020

GENERAL COMMENTS	This study has provided useful and important new information on CKD and its risk factors in the state of Punjab in India. I would like to suggest the following minor revisions before the manuscript can be accepted for publication. 1. Methods: Authors state that a subset of the original 5,127 individuals were selected for the study without mentioning the criterion used to select the subset. Authors should state how they selected this subset from the original sample. 2. Table 1. Authors have compared the means of the two populations for most of the parameters but used the medians to compare UACR. The authors should justify the reasons for this or follow uniformity in the comparisons. 3. The authors have concluded that the high prevalence of albuminuria in the study population cannot be explained by the traditional risk factors such as diabetes and hypertension and have suggested that there could be an environmental risk factor/s accounting for this. However the authors need to justify this claim further by addressing the following concerns. a. According to methods section diabetes has been diagnosed in individuals in Punjab based on a previous diagnosis of diabetes and has not been based on any laboratory tests. It is likely that this may have lead to gross under diagnosis of diabetes in the study population considering that the majority may have had limited access to testing or may not have had opportunistic screening for diabetes previously. If this is to be the case then the high prevalence of albuminuria could be partly explained by the missed cases of diabetes. b. CKD due to non-traditional causes have been reported from other parts of the world including Andrapradesh in India, Sri Lanka and countries in the Mesoamerican region. The whole mark of CKD in these areas is the lack of albuminuria in the early disease. The authors need to compare their findings with other CKDu endemic areas and need to explain the reasons for high

	albuminuria detected in this CKD population in Punjab compared to individuals with CKD due to non-traditional causes from other regions. 4. The authors have used various statistical methods to compare and contrast the two populations from Punjab and US. I feel they need to provide a more detailed account of these methods in the methods section so that the readers will be able to understand these better.

REVIEWER	Tazeen Jafar Duke-NUS Medical School Singapore
REVIEW RETURNED	27-Jun-2020

GENERAL COMMENTS	The manuscript by Bragg-Gresham et al is an interesting comparison of representative surveys, of about 2000 subjects in Punjab India (2014-2015) and over 5000 in NHANES (2014-2015). They found that an alarmingly high prevalence of albuminuria (30 mg/g) with 1 in 2 affected in Punjab, compared to 1 in 10 in the US, despite a younger population in Punjab. Was it urban or rural Punjab? Although a high prevalence of albuminuria in the native Indian population is expected as this population is at high risk of vascular disease, the estimates reported in the study are strikingly higher than previous reports from India. How was ACR measured in Punjab? Please clarify the method of data collection, transfer from the field site, reagents used for urine albumin, and creatinine. Was it based on semi-quantitative testing? Was the laboratory accredited to international standards? Please share any reproducibility data on the measurements. Could the differences be due to variation in measurement protocols for albuminuria between the two surveys? Did the investigators repeat measurements at least on a subset? The Punjab sample was significantly lighter and many were malnourished. Although lean body mass is not reported, it is possible that the difference in ACR is due to differences in urine creatinine excretion. The latter is expected to be lower in thin individuals on vegetarian diets. If 24-hour urine creatinine is available the measurement could be corrected for that. Else, could the authors compare mean levels of urine albumin excretion (mg/ml) not corrected for urine creatinine in the 2 samples? Does the marked difference still persist? How were serum creatinine measurements standardized to IDMS in Punjab samples? Was the history of drug intake especially over the counter NSAIDs collected? Please provide 95% confidence intervals for the estimates presented in each cell in Table 2. Do the authors think that the diminished association of albuminuria with diabetes in Punjab compared to the US is because many of the former are undiagnosed and therefore underreported diabetes? Air pollution could certainly be a contributing factor to vascular disease and albuminuria. However, estimates of albuminuria from other reports from India (Trivedi H et al, Clin Kidney J 2016) although higher than in the Western population are about one-third of that reported in this study.

REVIEWER	David Wegman University of Massachusetts Lowell, USA
REVIEW RETURNED	02-Jul-2020

GENERAL COMMENTS	This is a relatively straight forward presentation seeking to understand population prevalence of CKD in Punjab, India and to compare findings to those from the NHANES population sample in the US. The presentation of the methods and results is clear and appropriate for the most part, and the information communicated well with the tables although the figures are much less helpful. The manuscript would benefit from some added detail and explanation to make it more valuable and useful in the evolving understanding of CKD globally. Methods: Advantage is taken of the NHANES population survey to identify basic characteristics of kidney function among US adults. An apparently similar population survey (STEPS) was done in Punjab over dates that were almost the same and the two samples covered the same age range. The multi-stage stratified sampling methodology behind NHANES is well documented and publicly available. Although the Punjab sample is described similarly the population used in addressing kidney function was only 40% of the original sample. The sampling method may still have maintained its representative character, but it would be valuable to state this explicitly, if true, and to address any concerns about the study sample, if not true. As is, the US sample is 0.0014% of the US population while the Punjab sample is 0.18% of that state's population (or 40% of that for the final sample). Recognizing that multistage stratified samples cannot appropriately be characterized by use of simple proportions, the magnitude of the sample size difference between the two geographical areas is notable. This would likely be unimportant, but population differences illustrated in Table 1 are barely commented upon. Average age difference between Punjab and the US is not surprising but one wonders if a representative sample is correct in suggesting the population of Punjab is so heavily male (58%). Further the unusual Punjab findings for albuminuria suggest some mention of sampling differences would be welcome. The authors reported that diabetes and hypertension were similarly defined but they should note if other variables in Table 1 are measured essentially the same way in NHANES and STEPS: e.g., smoking, serum creatinine, albuminuria, triglycerides, and cholesterol. Results/Discussion: The finding of quite high albuminuria in Punjab is striking. Had the authors not found something similar in their study in China it would be very worrisome. Even still, one has to wonder if there was a review and confirmation that this is not a laboratory or test-based finding. At least this possibility should be noted in the discussion. The examination of CKD according to KDIGO criteria notes that results are only one point in time and so KDIGO can't be formally invoked. Nonetheless this is still a reasonable approach to organizing kidney function variability in a population. The findings using these criteria are presented logically in Table 2. The risk categories however are transferred from that table to Figure 1 in a confusing manner as the KDIGO categories are restructured for

	the figure in an unexplained manner and are therefore more confusing than helpful. To examine differences in and the importance of common risk factors for CKD, results are organized from their modelling effort in Tables 3a-c. These also are not sufficiently described or presented when used in Figure 2. In characterizing the figure the authors note that crude results were adjusted by “demographics” alone and then by demographics and “other health measures”. This is an inadequate description of how the modeling was performed to provide evidence for the figure. The message is that the striking differences in crude findings are accounted for by population differences for eGFR (almost entirely due to demographic differences). What is meant by “demographics” is not explained but is likely age and sex. Differences in albuminuria are not affected much at all by the modeling accounting for demographic differences alone or demographics plus “other health measures” which measures are also unexplained. Given the impact of demographics on eGFR differences and the striking difference in the proportion of males in the Punjab population (noted above) as well as the somewhat younger Punjab population, a stratified analysis of some type would seem to be in order for sex and possibly age. Overall there are striking differences between Punjab and the US. Modeling has provided some insights but primarily have led the authors to focus on non-traditional causes of CKD in Punjab. This may, in fact, be correct although it seems unlikely that air pollution, alone, is the non-traditional factor while the other factors referenced, water and soil pollution, but the evidence for these in other settings has been poor documented. Further consideration of the unusual health measure differences should be discussed as differences are not unidirectional hence the model has limited explanatory value when incorporating “health measures” as in Figure 2 - (hypertension is much higher and CVD higher in Punjab while smoking, diabetes, obesity and cholesterol are lower). The discussion would be enhanced by more substantial exploration of what is known about kidney disease or other chronic conditions where albuminuria and eGFR are so strikingly discordant. Such a discussion should also better attend to the cross-sectional nature of this prevalence study. The authors are aware of the problem (listed in limitations) but the discussion points to looking for drivers of eGFR and albuminuria from differential exposures or unknown risk factors when these may equally be related to other diseases such as CVD. The authors should give consideration to a less dramatic and possibly misleading title. A few minor items should be addressed in any revised manuscript: • The summary of limitations is inadequate. There are limitations related to overrepresentation of males in the Punjab sample and possibly others. • Page 11, line 3, the number should be 3.6%, not 3.8% • Page 11, line 4, the number should be 8.8%, not 8.9% (although this may be a rounding issue

	• Page 16, line 39 refers to “patients” but these are not patients they are a population sample • Page 17, lin8 refers to “countries” but the authors have earlier noted that Punjab is not necessarily representative of India, nor do they intend it to be • Page 19, line 49 concerns the possible limitation of the STEPS sample being appropriately representative of Punjab due to “sample size” but, as noted above, the sample size is proportionally much larger than that for NHANES. There might be concern that the multistage stratified sample was not as “good” as NHANES but size alone seems an inappropriate concern for Punjab alone.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Reviewer Name

Eranga Wijewickrama

Institution and Country

Faculty of Medicine, University of Colombo, Sri Lanka

Please state any competing interests or state ‘None declared’:
None declared

Please leave your comments for the authors below. This study has provided useful and important new information on CKD and its risk factors in the state of Punjab in India. I would like to suggest the following minor revisions before the manuscript can be accepted for publication.

Thank you for your detailed and helpful comments on our manuscript. We have addressed each comment below and in the manuscript where appropriate.

1. Methods: Authors state that a subset of the original 5,127 individuals were selected for the study without mentioning the criterion used to select the subset. Authors should state how they selected this subset from the original sample.

\ We have now included in the methods section information pertaining to how both samples were selected. For Punjab, only the STEP 3 sample was used due to the fact that it was the only sample with kidney measures. It was chosen as a subsample due to financial constraints and created by selecting every 2nd individual contacted for STEP 1 and 2.
\ For US NHANES, the sample included all adults ages 18 or older with complete information on kidney labs. Missing information on kidney labs was minimal, so the largest restriction was based on age, to remove children. This was also done to align with the sample ages in the Punjab sample.

2. Table 1. Authors have compared the means of the two populations for most of the parameters but used the medians to compare UACR. The authors should justify the reasons for this or follow uniformity in the comparisons.

\ We have added the reasoning for using the median for ACR to the methods section and as a Footnote in Table 1. The distribution of ACR was highly skewed with a long tail at the highest values. For this reason it is not appropriate to compare the means, as we did with the other variables which were much more normally distributed.

3. The authors have concluded that the high prevalence of albuminuria in the study population cannot be explained by the traditional risk factors such as diabetes and hypertension and have suggested that there could be an environmental risk factor/s accounting for this. However the authors need to justify this claim further by addressing the following concerns.

a. According to methods section diabetes has been diagnosed in individuals in Punjab based on a previous diagnosis of diabetes and has not been based on any laboratory tests. It is likely that this may have led to gross under diagnosis of diabetes in the study population considering that the majority may have had limited access to testing or may not have had opportunistic screening for diabetes previously. If this is to be the case then the high prevalence of albuminuria could be partly explained by the missed cases of diabetes.

\ The reviewer makes a good point. However, we have reviewed the definition for diabetes used in the Punjab data. Fasting glucose was considered in determining if an individual was diabetic. We have revised the methods section to reflect this. “Diabetes was defined by presence of any of the following: being told by a doctor they had diabetes, taking medication for diabetes (including medication from traditional healers in India), or fasting glucose > 126 mg/dl.”

b. CKD due to non-traditional causes have been reported from other parts of the world including Andhra Pradesh in India, Sri Lanka and countries in the Mesoamerican region. The whole mark of CKD in these areas is the lack of albuminuria in the early disease. The authors need to compare their findings with other CKDu endemic areas and need to explain the reasons for high albuminuria detected in this CKD population in Punjab compared to individuals with CKD due to non-traditional causes from other regions.

\ In thinking about this further, we expand on our speculation that the high prevalence of albuminuria in Punjab could be related to metabolic syndrome (known to be associated with endothelial dysfunction, vascular disease that could lead to albuminuria). Insulin resistance and visceral adiposity are common in India. The high prevalence of premature cardiovascular disease and hypertension can be accompanied by albuminuria from vascular dysfunction or damage, leading to disruption of the glomerular filtration barrier. Environmental factors such as air pollution (highly prevalent in that part of the world), is associated with both endothelial dysfunction and low grade inflammation with resultant albuminuria. Whether environmental causes other than air pollution are also associated with albuminuria, would be speculative at best, but plausible. Future studies would be well advised to investigate this matter in greater detail both using body composition measurements, vascular function and toxicological studies as well as those based on kidney biopsies, as part of a well-crafted prospective research protocol. We now modify our text in the discussion to accommodate the potential explanations as described above, for high prevalence of albuminuria in Punjab, India. We have also added several salient references.

4. The authors have used various statistical methods to compare and contrast the two populations from Punjab and US. I feel they need to provide a more detailed account of these methods in the methods section so that the readers will be able to understand these better.

\ The methods section has been expanded to more thoroughly reflect statistical analyses conducted.

Reviewer: 2

Reviewer Name

Tazeen Jafar

Institution and Country

Duke-NUS Medical School Singapore

Please state any competing interests or state ‘None declared’:
None

Please leave your comments for the authors below

The manuscript by Bragg-Gresham et al is an interesting comparison of representative surveys, of about 2000 subjects in Punjab India (2014-2015) and over 5000 in NHANES (2014-2015). They found that an alarmingly high prevalence of albuminuria (30 mg/g) with 1 in 2 affected in Punjab, compared to 1 in 10 in the US, despite a younger population in Punjab.

Was it urban or rural Punjab?

\ The sample contained a representative sample of individuals from both urban and rural Punjab, based on the 2011 Census.

Although a high prevalence of albuminuria in the native Indian population is expected as this population is at high risk of vascular disease, the estimates reported in the study are strikingly higher than previous reports from India.

\ We agree and the consequences could be catastrophic if disease progresses in this population and the community needs to be made aware.

How was ACR measured in Punjab? Please clarify the method of data collection, transfer from the field site, reagents used for urine albumin, and creatinine. Was it based on semi-quantitative testing? Was the laboratory accredited to international standards? Please share any reproducibility data on the measurements.

\ We have added more detail to the methods concerning the methods for sample data collection.
\ For Punjab:
o Collection of blood and urine samples was done at mornings of the next day when participants had fasted overnight.
o Collection of all the biochemical tests was at the household level.
o Collected blood samples were centrifuged using a mini-centrifuge and the separated serum was stored in ice boxes. Collected samples were transferred daily to a nearest public health facility for -20ºC storage. From there samples were transported to the central laboratory at PGIMER on a weekly basis.
o Urine albumin-to-creatinine ratio was performed as a point-of-care field test using the validated URS 2AC strip that tests for 2 parameters microalbumin and creatinine (Biosense Technologies, Thane,

Maharashtra, India).
o Laboratory measurements of creatinine were made on Modular P 800 auto-analyzer (Roche Diagnostics, Germany) using commercially available kits (Roche Diagnostics, Germany)
o Calibration of the instruments and reliability testing of assays, was performed by the biochemistry laboratory at PGIMER, Chandigarh.

Could the differences be due to variation in measurement protocols for albuminuria between the two surveys?

\ We do not feel this large difference in estimates of albuminuria could be driven by differences in protocol, as standard protocols were used at each site. The prevalence of albuminuria was estimated to be 8.9% in the US sample compared to 46.7% in the Punjab. While inter- laboratory differences could cause some variation, this difference seems too large to be able to be explained by this factor alone.

Did the investigators repeat measurements at least on a subset?

\ A proportion of samples in the Punjab study were repeat tested in the central lab of PGIMER, Chandigarh. The point of care urine ACR testing was also validated by the central lab.
\ In the US NHANES sample: Contract laboratories randomly perform repeat testing on 2% of all specimens. (http://data.nber.org/nhanes/2015-2016/ALB_CR_I.htm) This has been added to the methods section.

The Punjab sample was significantly lighter and many were malnourished. Although lean body mass is not reported, it is possible that the difference in ACR is due to differences in urine creatinine excretion. The latter is expected to be lower in thin individuals on vegetarian diets. If 24-hour urine creatinine is available the measurement could be corrected for that. Else, could the authors compare mean levels of urine albumin excretion (mg/ml) not corrected for urine creatinine in the 2 samples? Does the marked difference still persist?

\ While differences in urinary creatinine excretion may exist between populations (and within populations) due to dietary or muscle mass or other factors (renal tubular secretion or non-renal secretion), the ACR was expressed in mg/g of creatinine in both the Punjab and US. It is therefore unlikely for this phenomenon to account for the difference in ACR between the two populations.
\ However, in response the reviewer's comment, we have analyzed urine albumin values without the correction for urine creatinine and added this information along with serum creatinine to Table 1. All differences persisted with $p<0.0001$:
o Urine Albumin (mg/mmol): Punjab = 0.2 (0.03) vs. US = 0.07 (0.002)
o Urine Albumin (mg/dL): Punjab = 2.3 (0.3) vs. US = 0.7 (0.02)
o Urine Creatinine (µmol/L): Punjab = 7,242 (265) vs. US = 9,275 (292)
o Urine Creatinine (mg/dl): Punjab = 81.9 (3.0) vs. US = 104.9 (3.3)

How were serum creatinine measurements standardized to IDMS in Punjab samples?

\ The Central PGIMER biochemistry lab uses a serum creatinine assay that is standardized to the IDMS standard.

Was the history of drug intake especially over the counter NSAIDs collected?

\ Information concerning all medication used within the past 30 days is available in the US NHANES database, but only information on medications being currently taken for hypertension, diabetes, and cardiovascular diseases were obtained in the Punjab STEPS survey. Unfortunately we are not able to look at prior NSAID use. Future studies should collect information on over the counter and traditional medications prescribed by indigenous practitioners, as these could in part be linked to kidney damage with resultant albuminuria.

Please provide 95% confidence intervals for the estimates presented in each cell in Table 2.

\ The 95% CI's have been added to Table 2.

Do the authors think that the diminished association of albuminuria with diabetes in Punjab compared to the US is because many of the former are undiagnosed and therefore underreported diabetes?

\ Because diabetes was defined very similarly between the two countries (diagnosis, medication, or lab indication) we do not feel there should be any underreporting in either country.
\ As we speculate in response to reviewer 1, prediabetes in association with visceral adiposity in India could be one of the reasons for higher prevalence of albuminuria. Air pollution and higher prevalence of cardiovascular disease in general, could be other potential explanations, in addition to unknown environmental (soil/food/water) toxins. We have added references to support this argument.

Air pollution could certainly be a contributing factor to vascular disease and albuminuria. However, estimates of albuminuria from other reports from India (Trivedi H et al, Clin Kidney J 2016) although higher than in the Western population are about one-third of that reported in this study.

\ This report by Trivedi H et al., the reviewer alludes to, describes the prevalence of albuminuria in the state of Gujarat, India. This was based on a voluntary sample of participants screened during a World Kidney Day Screening Camp. It is important to note that they excluded participants with known diabetes, stone diseases, hypertension, kidney/liver/cardiac disease, hepatitis, HIV, transplant recipients, pregnant women and those < 18 years of age. These exclusions would have naturally removed many individuals with albuminuria and, while from a different region of India, it very likely underestimates the true burden of albuminuria in that area. Furthermore, Trivedi et al utilized urine dipstick to determine albuminuria, while we used ACR, a more accurate correlate of 24 hour urinary albumin excretion. Nevertheless we are struck by their estimate of 13.8%, which despite the exclusions, appears higher than the US general population, suggesting that had they been more inclusive in their sampling technique, the prevalence of albuminuria in their study would potentially have been higher.
\ We thank the reviewer for pointing to this work. We have incorporated it into the discussion as further evidence of high prevalence of albuminuria in India.

Reviewer: 3

Reviewer Name

David Wegman

Institution and Country

University of Massachusetts Lowell, USA

Please state any competing interests or state ‘None declared’:
None declared

Please leave your comments for the authors below:

This is a relatively straight forward presentation seeking to understand population prevalence of CKD in Punjab, India and to compare findings to those from the NHANES population sample in the US. The presentation of the methods and results is clear and appropriate for the most part, and the information communicated well with the tables although the figures are much less helpful. The manuscript would benefit from some added detail and explanation to make it more valuable and useful in the evolving understanding of CKD globally.

Methods: Advantage is taken of the NHANES population survey to identify basic characteristics of kidney function among US adults. An apparently similar population survey (STEPS) was done in Punjab over dates that were almost the same and the two samples covered the same age range. The multi-stage stratified sampling methodology behind NHANES is well documented and publicly available. Although the Punjab sample is described similarly the population used in addressing kidney function was only 40% of the original sample. The sampling method may still have maintained its representative character, but it would be valuable to state this explicitly, if true, and to address any concerns about the study sample, if not true.

\ We thank the reviewer for pointing out that we should describe how the STEPS 3 sample was drawn. This has been added to the methods section. As described above for Reviewer #1, who had the same question: “For Punjab, only the STEP 3 sample was used due to the fact that it was the only sample with kidney related laboratory testing. It was chosen as a subsample due to financial constraints and created by selecting very 2nd individual contacted for STEP 1 and 2.” It is also important to note that sampling weights were employed that were specific to the STEP 3 sample.

As is, the US sample is 0.0014% of the US population while the Punjab sample is 0.18% of that state’s population (or 40% of that for the final sample). Recognizing that multistage stratified samples cannot appropriately be characterized by use of simple proportions, the magnitude of the sample size difference between the two geographical areas is notable.

\ This is an interesting way to compare the two samples. We have just calculated the following values, which are similar to yours for the US (0.0015%), but must smaller for the Punjab (0.0072%). We employed an estimate based on approximately 30 million residents in the Punjab, as reported in their 2020 census. Based on the sampling techniques we do not find the difference in samples sizes problematic.

This would likely be unimportant, but population differences illustrated in Table 1 are barely commented upon. Average age difference between Punjab and the US is not surprising but one wonders if a representative sample is correct in suggesting the population of Punjab is so heavily male (58%). Further the unusual Punjab findings for albuminuria suggest some mention of sampling differences would be welcome. The authors reported that diabetes and hypertension were similarly defined but they should note if other variables in Table 1 are measured essentially the same way in NHANES and STEPS: e.g., smoking, serum creatinine, albuminuria, triglycerides, and cholesterol.

\ Thank you for pointing out the need for inclusion of more description of the results and methods.

Checking the Indian census from 2011 (www.census2011.co.in), it appears at that time 53% of the population were male. While this is slightly lower than our sample estimate, the Punjab study was run meticulously and should represent the population at the time of sampling. Based on the reviewer's comments and those of reviewer #1, we have also added more description of the laboratory measures and handling of samples in the Punjab. The current study team was involved in study design for the sampling and storage of samples and does not feel any differences from those in the NHANES sample should be driving the large differences we are seeing.

Results/Discussion: The finding of quite high albuminuria in Punjab is striking. Had the authors not found something similar in their study in China it would be very worrisome. Even still, one has to wonder if there was a review and confirmation that this is not a laboratory or test-based finding. At least this possibility should be noted in the discussion.

\ This possibility has been added to the discussion under limitations.

The examination of CKD according to KDIGO criteria notes that results are only one point in time and so KDIGO can't be formally invoked. Nonetheless this is still a reasonable approach to organizing kidney function variability in a population. The findings using these criteria are presented logically in Table 2. The risk categories however are transferred from that table to Figure 1 in a confusing manner as the KDIGO categories are restructured for the figure in an unexplained manner and are therefore more confusing than helpful.

\ We appreciate knowing that you found the presentation of these numbers confusing. We have added a footnote to the table to explain the shading, which corresponds directly with the shading in Figure 1, and hope the parallelism will be clearer now.

To examine differences in and the importance of common risk factors for CKD, results are organized from their modelling effort in Tables 3a-c. These also are not sufficiently described or presented when used in Figure 2. In characterizing the figure the authors note that crude results were adjusted by "demographics" alone and then by demographics and "other health measures". This is an inadequate description of how the modeling was performed to provide evidence for the figure. The message is that the striking differences in crude findings are accounted for by population differences for eGFR (almost entirely due to demographic differences). What is meant by "demographics" is not explained but is likely age and sex. Differences in albuminuria are not affected much at all by the modeling accounting for demographic differences alone or demographics plus "other health measures" which measures are also unexplained.

\ A footnote has been added to define demographics (age and sex) in the figure. Also, more description has been added to the results section and explanation in the discussion. Your interpretation was correct.

Given the impact of demographics on eGFR differences and the striking difference in the proportion of males in the Punjab population (noted above) as well as the somewhat younger Punjab population, a stratified analysis of some type would seem to be in order for sex and possibly age.

\ A sensitivity analysis has been run, stratifying all 3 outcome models by sex to ensure that no differences were observed in the direction of associations and that they sample comparisons (Punjab vs. US) did not change. While the magnitude of associations did change slightly based on sex, no large or significant differences were seen. I have added this as a sentence in the methods and the

results.

Overall there are striking differences between Punjab and the US. Modeling has provided some insights but primarily have led the authors to focus on non-traditional causes of CKD in Punjab. This may, in fact, be correct although it seems unlikely that air pollution, alone, is the non-traditional factor while the other factors referenced, water and soil pollution, but the evidence for these in other settings has been poor documented.

\ We thank the reviewer for suggesting that we think about these differences some more. We have therefore incorporated some further thoughts about the differences observed in albuminuria in particular, and the lower prevalence of low eGFR in the Punjab as compared to the US. Please see our response to reviewer 1 in particular, on the same issue.

Further consideration of the unusual health measure differences should be discussed as differences are not unidirectional hence the model has limited explanatory value when incorporating “health measures” as in Figure 2 - (hypertension is much higher and CVD higher in Punjab while smoking, diabetes, obesity and cholesterol are lower). The discussion would be enhanced by more substantial exploration of what is known about kidney disease or other chronic conditions where albuminuria and eGFR are so strikingly discordant. Such a discussion should also better attend to the cross-sectional nature of this prevalence study. The authors are aware of the problem (listed in limitations) but the discussion points to looking for drivers of eGFR and albuminuria from differential exposures or unknown risk factors when these may equally be related to other diseases such as CVD. The authors should give consideration to a less dramatic and possibly misleading title.

\ The reviewer raises an important issue about the discordance observed in the albuminuria versus lower eGFR between India and the US. We believe that this could also in part be due to the epidemiologic transition that is occurring in countries such as India, where early evidence of kidney damage but lower prevalence of low eGFR defined kidney disease or end stage kidney disease, may be the result of higher death rates among the younger population from premature cardiovascular disease, so while early kidney disease evidenced by albuminuria is more common, but prevalence of later stages of kidney disease is lower. We have added these considerations to the discussion.

A few minor items should be addressed in any revised manuscript:

\ Thank you for pointing these out. Corrections have been made where appropriate.

• The summary of limitations is inadequate. There are limitations related to overrepresentation of males in the Punjab sample and possibly others. After looking at the census in the Punjab, our percentage of males is not markedly higher.
• Page 11, line 3, the number should be 3.6%, not 3.8%
• Page 11, line 4, the number should be 8.8%, not 8.9% (although this may be a rounding issue)
• Page 16, line 39 refers to “patients” but these are not patients they are a population sample
• Page 17, lin8 refers to “countries” but the authors have earlier noted that Punjab is not necessarily representative of India, nor do they intend it to be
• Page 19, line 49 concerns the possible limitation of the STEPS sample being appropriately representative of Punjab due to “sample size” but, as noted above, the sample size is proportionally much larger than that for NHANES. There might be concern that the multistage stratified sample was not as “good” as NHANES but size alone seems an inappropriate concern for Punjab alone.

VERSION 2 – REVIEW

REVIEWER	Eranga Wijewickrama Faculty of Medicine, University of Colombo, Sri Lanka
REVIEW RETURNED	13-Aug-2020

GENERAL COMMENTS	The authors have addressed my concerns in a satisfactory manner.

REVIEWER	Tazeen Jafar Duke-NUS Medical School Singapore
REVIEW RETURNED	10-Aug-2020

GENERAL COMMENTS	Overall the revisions are satisfactory. However, please state in the limitations that some variability in albuminuria levels (although corrected for spot creatinine excretion) may be possible as the population in Punjab were more likely to be malnourished. (Of note the tubular secretion of creatinine may in fact be greater in people with extreme malnutrition- needs further study).

REVIEWER	David H Wegman University of Massachusetts Lowell, USA
REVIEW RETURNED	03-Aug-2020

GENERAL COMMENTS	1. It would be helpful to provide the actual modeling results presented in Figure 2 in a supplementary table or tables. 2. The added footnote to Figure 2 should identify the variables in the final model. Although most of the PR change occurs with demographic adjustment alone, the added information would be helpful. Did authors, in fact, include a variable for BMI and one for obesity category - seems unnecessary? 3. The limitation in using test strips over direct measurement of albuminuria (as used in the NHANES comparison) should be noted among the limitations of the study. The authors reference a validation for use of the strips but there is <72% specificity for the important outcome (albuminuria). While it is likely this would not have "created" the findings it is a limitation in the study. 4. The KDIGO-based figure (Figure 1) uses shaded colors of grey. For the on-line version of the publication, assuming no added cost, it would be good to add the KDIGO colors to enhance readability.

VERSION 2 – AUTHOR RESPONSE

Reviewer: 3
Reviewer Name: David H Wegman
Institution and Country: University of Massachusetts Lowell, USA
Please state any competing interests or state ‘None declared’: None declared

Please leave your comments for the authors below:
Thank you again for your comments that have helped to significantly improve our manuscript. Below are the point-by-point responses to your comments:

1. It would be helpful to provide the actual modeling results presented in Figure 2 in a supplementary table or tables.

\ These model results have been added as Supplemental Tables 1 - 3. Note, the results of the fully adjusted model were already included in Table 3 for each marker of kidney function (low eGFR, Albuminuria, or either indicator of CKD), so we did not repeat these in the Supplemental Tables. We have added a new row to Table 3 for each marker that contains the prevalence ratio comparing the US to the Punjab for these fully adjusted models, which match the prevalence ratio shown in Figure 2 for the fully adjusted model.

2. The added footnote to Figure 2 should identify the variables in the final model. Although most of the PR change occurs with demographic adjustment alone, the added information would be helpful. Did authors, in fact, include a variable for BMI and one for obesity category - seems unnecessary?

\ We have now listed out all the variables in the footnote and also direct the reader to Supplemental Tables 1 - 3 for more details. Obesity was actually run with both parameterizations (as BMI categories and continuous) in separate models. We gave both results for the BMI estimates, but the other adjustments presented came from the models with continuous BMI. We have added that clarification to the methods section and as a footnote as well.

3. The limitation in using test strips over direct measurement of albuminuria (as used in the NHANES comparison) should be noted among the limitations of the study. The authors reference a validation for use of the strips but there is <72% specificity for the important outcome (albuminuria). While it is likely this would not have "created" the findings it is a limitation in the study.

\ We agree and have now added this as a limitation in the discussion.

4. The KDIGO-based figure (Figure 1) uses shaded colors of grey. For the on-line version of the publication, assuming no added cost, it would be good to add the KDIGO colors to enhance readability.

\ We agree that color would make both the KDIGO table and figure much easier to interpret. We have looked into the charges and it appears there is no charge for color. We have therefore added color to both the table and figure in the manuscript.