PEER REVIEW HISTORY

This paper was submitted to a another journal from BMJ but declined for publication following peer review. The authors addressed the reviewers' comments and submitted the revised paper to BMJ Open. The paper was subsequently accepted for publication at BMJ Open.

(This paper received three reviews from its previous journal but only two reviewers agreed to published their review.)

ARTICLE DETAILS

TITLE (PROVISIONAL)	The relationship between serum uric acid and clustering of cardiovascular disease risk factors, renal disorders among Shanghai population: a multi-center and cross-sectional study
AUTHORS	Tao, Min; Pi, Xiaoling; Ma, Xiaoyan; Shi, Yingfeng; Zhang, Yuzhen; Gu, Hongwei; Chi, Yongbin; Zhuang, Shougang; Liu, Na

VERSION 1 – REVIEW

REVIEWER	Cicero AF Italy
REVIEW RETURNED	21-Sep-2018

GENERAL COMMENTS	I've read with attention the paper of Liu et al. that is potentially of interest. The methodology applied is overall correct, the results reliable and adequately discussed. I've only some minor concerns/suggestions: - Abstract: it should contain some quantitative data, while the conclusion should be shortened - A STROBE chart has to be added - The discussion is a bit unfocused and should consider to cite some papers from Borghi C et al. - The study limitation should be also included in the discussion section

REVIEWER	Piotr Choręza Deparment of Statistics, School of Pharmacy with the Division of Laboratory Medicine in Sosnowiec, Medical University of Silesia in Katowice, Sosnowiec
REVIEW RETURNED	13-Nov-2018

GENERAL COMMENTS	The relationship between serum uric acid concentration and prevalence of cardiovascular risk factors is well established. Therefore a substantial part of the results and conclusions duplicates previous studies. The new, interesting observation is the gender differences in the correlation between SUA and DM, as well as the gender differences in the association between hyperuricemia and clustered cardiovascular risk factors presented in Table 6. Big sample size is the significant advantage of the study. Unfortunately, the discussion is superficial and in my opinion, it should be expanded. Obtained Person's correlation coefficients are low, I refer to base the discussion on the ORs and

| | 95% CI.
This study have some limitations, eg. lack of information about used pharmacotherapy; it must be noted, that some drugs, ex. thiazide diuretics may increase kidneys' burden significantly. Moreover, lack of information about lifestyle, addictions and diet, especially fructose intake, is a serious deficiency.

Comments:
1. Page No 5, lines 44-45.
First of all, patients treated with the use of xanthine oxidase inhibitors (eg. allopurinol, febuxostat) should be excluded. Secondary, there is: "uric acid (UA)-lowing drugs", should be: "xanthine oxidase inhibitors".
2. Page No 7, section: Data collection
There should be information about producers of the laboratory reagents that were used.
3. Page No 7, section: Statistical analysis.
There is lack of information about statistical tests that were used to assess quantitative variables' distribution and the homogeneity of variance (that is one of the ANOVA's assumptions). In ANOVA we are testing if groups' means differs each other, but not "differences between continuous variables", as Authors wrote at Page No 7, line 53. Moreover, the post-hoc tests should be done if ANOVA's results show differences between the groups.
4. Page No 9, lines 23-35 & Table 2.
Person's correlation coefficients between SUA and each parameter shows very week correlation between variables. That results presented in Table 2, especially for males does not justified the conclusions presented on the page No 9. Moreover, coefficients of determination of the models (r-squared) are low. Obtained p values below 0.001 are, the more likely, the effect of the big sample size.
I refer to use the multivariate regression models rather than linear regression models.
5. Table 1
Patients' waist and HOMA-IR should be presented additionally. Did the all of the variables have a normal distribution?
6. Page No 15, References.
Authors cited 52 articles, but only 9 of them have been published 2013 – up to date. 15 articles were published before 1999 and 9 of them, before 1995. So collected bibliography is nowadays unacceptable. |

VERSION 1 – AUTHOR RESPONSE

Reviewer 1
1. Abstract: it should contain some quantitative data, while the conclusion should be shortened.
We accepted the reviewer's suggestion and added some quantitative data into abstract. After multiple adjustment, hyperuricemia positively connected with obesity (Male odds ratio (OR)=3.115, P<0.001; Female OR=3.755, P<0.001), hypertension (Male OR=1.290, P<0.001; Female OR=1.287, P=0.006), dyslipidemia (Male OR=2.503, P<0.001; Female OR=3.675, P<0.001), chronic kidney disease (Male OR=6.962, P<0.001; Female OR=11.508, P<0.001), nephrolithiasis (Male OR=1.480, P<0.001; Female OR=1.239, P=0.042), but negatively connected with diabetes mellitus ((Male OR=0.205, P<0.001; Female OR=0.514, P<0.001)). Women had a stronger association between hyperuricemia and clustered cardiovascular disease risk factors (CRFs), CKD than men.
The conclusion has been shortened. In Shanghai population, concomitant with the elevated level of SUA, the prevalence of CRFs and renal diseases was rising. Hyperuricemia was significantly associated with CRFs and renal disorders, especially in females.

2. A STROBE chart has to be added.
We accepted the reviewer's suggestion and added the STROBE chart.

3. The discussion is a bit unfocused and should consider to cite some papers from Borghi C et al.
We accepted the reviewer's suggestion and further focused our discussions. We focused our discussion on the relationship between obesity and SUA, hypertension and SUA, diabetes mellitus and SUA, CVD and SUA, CKD and SUA. The study limitation has also been discussed. Professor Borghi carried out many convictive and scientific analysis on the relationship between SUA and CRFs. We observed that more CRFs individuals had the higher ORs of hyperuricemia in both genders. This was consistent with Borghi C's studies, which supported an independent association between SUA and CVDs.1,2

4. The study limitation should be also included in the discussion section.
We accepted the reviewer' comment and added the study limitation in the revised manuscript. First, our study was a cross-section observation and the results could not establish causative relationships between hyperuricemia and CRFs clustering and renal diseases. Second, the data were from three medical centers' databases that lacked details in waist, HOMA-IR, smoking, drinking, lifestyles, diet and pharmacotherapy, which might affect the deviations of some clinical outcomes. Nonetheless, the strengths of our study included its strict exclusion criteria based on medical histories and laboratory findings. And we conducted a multi-center study with large sample size which ensured sufficient results. The relationship was analyzed in both sexes and we got a solid conclusion about the differences between men and women.

Reviewer 2
1. Page No 5, lines 44-45. First of all, patients treated with the use of xanthine oxidase inhibitors (eg. allopurinol, febuxostat) should be excluded. Secondary, there is: "uric acid (UA)-lowing drugs", should be: "xanthine oxidase inhibitors".
We accepted the reviewer's suggestion and excluded the subjects treated with xanthine oxidase inhibitors (eg. allopurinol, febuxostat) in the final enrolled population. As such, we added this exclusion criteria into Study Population Section. Moreover, we have changed the description "uric acid (UA)-lowing drugs" into "xanthine oxidase inhibitors" in the Primary Outcomes Section.

2. Page No 7, section: Data collection. There should be information about producers of the laboratory reagents that were used.
We accepted the reviewer's suggestion and added the information about the producer of the reagents that were used in our laboratory. They were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

3. Page No 7, section: Statistical analysis. There is lack of information about statistical tests that were used to assess quantitative variables' distribution and the homogeneity of variance (that is one of the ANOVA's assumptions). In ANOVA we are testing if groups' means differs each other, but not "differences between continuous variables", as Authors wrote at Page No 7, line 53. Moreover, the post-hoc tests should be done if ANOVA's results show differences between the groups.
We accepted the reviewer's suggestion and added the information about statistical tests. The distribution and homogeneity of variance were tested. The continuous variables are reported in means±SD and categorical variables are presented in percentages. In case of nonparametric data distribution medians with inter quartile range (IQR) are presented. The univariate analysis of variance (ANOVA) was used to measure the data among the groups' means or a Kruskal-Wallis test in case of nonparametric data distribution. Differences between groups for proportions were tested with a chi-square test. The results showed differences between the groups, the post-hoc tests have been done and marked in Table 1 (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs. the group of Q1). As for the post-hoc

test, we used least significance difference (LSD) test if the variance was homogeneous, and we used Tamhane's T2 test if not.

4. Page No 9, lines 23-35 & Table 2. Person's correlation coefficients between SUA and each parameter shows very week correlation between variables. That results presented in Table 2, especially for males does not justified the conclusions presented on the page No 9. Moreover, coefficients of determination of the models (r-squared) are low. Obtained p values below 0.001 are, the more likely, the effect of the big sample size. I refer to use the multivariate regression models rather than linear regression models.

We accepted the reviewer's suggestion and use the multiple linear regression models. We added the table in the place of Table 3. All of the variables (SUA, age, BMI, SBP, DBP, FPG, TC, TG, HDL-C, LDL-C, eGFR) have normal distributions. Thus, Pearson's correlation was used among these variables. If Person's correlation analysis was statistically significant, multiple linear regression analysis was performed. We analyzed the multiple collinearity by calculating the correlation coefficient matrix, tolerance and variance inflation factor of independent variables. Multicollinearity analysis showed that SBP and DBP highly correlated with each other, so do TC and LDL-C. And backward elimination was adopted for multiple linear regression to identify independent variables which have most impact on dependent variables. Finally, independent variables DBP and LDL-C were removed in male and in total. Independent variables DBP, LDL-C and age were removed in female. The results of multiple linear regression analysis in Table 3 showed that adjusting for various factors, serum uric acid was still positively correlated with BMI, SBP, TC, TG, negatively correlated with age, FPG, HDL-C and eGFR in males (all P values<0.001). Serum uric acid was positively correlated with BMI, SBP, TC, TG, negatively correlated with FPG, HDL-C and eGFR in females (all P values<0.001, except FPG P values=0.002).

5. Table 1. Patients' waist and HOMA-IR should be presented additionally. Did the all of the variables have a normal distribution?

Waist circumference can assess visceral adiposity. HOMA-IR (homeostasis model assessment of insulin resistance) are good evaluations for insulin resistance. Our data were from three medical centers' databases, and we failed to collect those information. Although we were lack of those two indexes, the models we established were stable. Moreover, serum uric acid was significantly associated with obesity and diabetes mellitus.

The distribution and homogeneity of variance were tested by SPSS statistics version 20.0. The results showed that all of the variables (SUA, age, BMI, SBP, DBP, FPG, TC, TG, HDL-C, LDL-C, eGFR) have normal distributions.

6. Page No 15, References. Authors cited 52 articles, but only 9 of them have been published 2013 – up to date. 15 articles were published before 1999 and 9 of them, before 1995. So collected bibliography is nowadays unacceptable.

We have updated the references. From 2013 to present, 33 of relevant articles have been published. 10 articles were published from 2012 to 2007, 8 articles were published from 2000 to 2006, only 3 of them were published before 1999.

VERSION 2 – REVIEW

REVIEWER	Arrigo Francesco Giuseppe Cicero University of Bologna, Italy
REVIEW RETURNED	04-Dec-2018

GENERAL COMMENTS	The authors have considered the reviewers' suggestiona and improved the paper accordingly. I've no further comment on it.

REVIEWER	Piotr Choręza Department of Statistics, Department of Instrumental Analysis, School of Pharmacy with the Division of Laboratory Medicine in Sosnowiec, Medical University of Silesia, Poland; 30 Ostrogórska Str., Sosnowiec 41-200
REVIEW RETURNED	13-Dec-2018

GENERAL COMMENTS	Comments: 1. In the response letter to the first review Authors wrote in the first point, that patients treated with the use of the xanthine oxidase inhibitors were excluded. So, the number of patients enrolled to the study was 27176. It is a surprising, because the same number of patients was enrolled in the first version (without additional excluding criteria). Moreover, Tables No 1, 2, 4, 5, 6 and 7 present the same data, as tables in previous version, that is unlikely (especially in case of baseline characteristics). I would like to know, how many patients, in the whole group (N = 27176?) was treated with the use of xanthine oxidase inhibitors and how many of them were excluded. Please check, if the presented data is correct. 2. Page No 8 – Section: Statistical analysis Statistical methodology is well chosen, but in my opinion the description is a bit chaotic (eg. Page No 9, Lines 169 – 170; that fragment suggests that that Authors performed post-hoc tests for the analysis of the qualitative data).

VERSION 2 – AUTHOR RESPONSE

Reviewer 2

1. In the response letter to the first review Authors wrote in the first point, that patients treated with the use of the xanthine oxidase inhibitors were excluded. So, the number of patients enrolled to the study was 27176. It is a surprising, because the same number of patients was enrolled in the first version (without additional excluding criteria). Moreover, Tables No 1, 2, 4, 5, 6 and 7 present the same data, as tables in previous version, that is unlikely (especially in case of baseline characteristics). I would like to know, how many patients, in the whole group (N = 27176?) was treated with the use of xanthine oxidase inhibitors and how many of them were excluded. Please check, if the presented data is correct.

We accepted the reviewer's suggestion and checked the data again. 408 individuals who treated with xanthine oxidase inhibitors (eg. allopurinol, febuxostat) were excluded. Finally, there are 26768 participants in the final enrolled population. We re-analyzed our data. Tables No 1, 2, 3, 4, 5, 6 and 7 and Figure 1 have been replaced.

2. Page No 8 – Section: Statistical analysis
Statistical methodology is well chosen, but in my opinion the description is a bit chaotic (eg. Page No 9, Lines 169 – 170; that fragment suggests that that Authors performed post-hoc tests for the analysis of the qualitative data).

We accepted the reviewer's suggestion and re-described the statistical analysis section as follow.
We divided the subjects into gender-specific quartiles [males (M): Q1≤ 4.9, Q2: 5.0-5.9, Q3: 6.0-6.9, Q4 ≥ 7.0 mg/dL; females (F): Q1≤ 3.9, Q2: 4.0-4.9, Q3: 5.0-5.9, Q4 ≥ 6.0mg/dL] according to serum uric acid level. Distribution of variables was evaluated by the Kolmogorov Smirnov test and homogeneity of variance was assessed by the Levene test. The normal distributed data are reported in means±SD. Skewed or non-normal distributed data are presented in medians with inter quartile range (IQR). Categorical variables are showed in percentages. The univariate analysis of variance (ANOVA) was used to analyze the differences among groups' means in case of normal data distribution or after logarithmic normalization in case of skewed data (if appropriate). Kruskal-Wallis

test was used to analyze the differences among groups' medians in case of nonparametric data distribution. Differences between groups for proportions were tested with chi-square tests. If the results show differences between the groups, the post-hoc tests would be done. As for the post-hoc test of normally distributed data, we used least significance difference (LSD) test if the variance was homogeneous, and we used Tamhane's T2 test if not. The post-hoc test of non-normally distributed data was compared using Kruskal-Wallis test. As for the post-hoc test of categorical variables, we used chi-square tests. Correlations were Pearson's or Spearman's depending on the distribution of the data. In the present study, SUA, age, BMI, SBP, DBP, FPG, TC, TG, HDL-C, LDL-C, eGFR were normally distributed. Thus, Pearson's correlation was used among these variables. If Pearson's correlation analysis was statistically significant, multiple linear regression analysis was performed to determine the association of SUA with various independent variables. We analyzed the multiple collinearity by calculating the correlation coefficient matrix, tolerance and variance inflation factor of independent variables. Multivariable logistic regression analysis (unadjusted and full-adjusted) was used to calculate the odds ratio for hyperuricemia according to different status of clinical parameters. Furthermore, multivariable logistic regression analysis (multiple adjusted models) was used to examine the association between related diseases and the SUA categories of Q2 or greater compared to the lowest SUA category. The association between hyperuricemia and clustered CVD risk factors had been calculated. Statistical analyses were performed by IBM SPSS statistics version 20.0 (SPSS, Chicago, IL, USA). Statistical significance was set at P-values of <0.05.

VERSION 3 – REVIEW

REVIEWER	Piotr Choręza Department of Statistics, Department of Instrumental Analysis School of Pharmacy with the Division of Laboratory Medicine in Sosnowiec, Medical University of Silesia in Katowice, Poland
REVIEW RETURNED	16-Jan-2019

GENERAL COMMENTS	The Authors have considered the reviewers' suggestions. That paper have some limitations, but they are cleary presented. In my opinion the article is fine, and after the proofreading, it may be published.