

LETTER TO THE EDITOR



Reply to 'Methodological and statistical questions regarding a study on hair nickel and chromium'

Dear Editor

Thank you very much for the opportunity to submit a response letter. We are also thankful to Dr. Rakhshan for his interest in our published study [1]. Dr. Rakhshan brought up some questions with regard to our article and we responded to him. His questions and comments helped us to strengthen our article.

Methodology

1. As you correctly pointed it, 1 µg/g is equal to 1 ppm. The context in the method section must be presented as ppm not ppb. Therefore, the correct form of this sentence is 'The resulted data were reported as the mean ± SE in ppm.'
2. The presented study is not a case-control study (as we mistakenly stated) and also not a clinical trial (as the letter sender pointed). This type of study is called 'prospective cohort study'. Type of the study was correctly stated in our proposal (Persian version at: http://pajouhan.mubabol.ac.ir/webdocument/load.action?web-document_code=1000&masterCode=11003049). The correct form of this sentence is 'This prospective cohort study was performed on 52 subjects ...'.
3. There are various routes for entering the metals into the body. As you pointed, definitely we had other unaccounted sources of the metal entrance, but we were not able to exclude all of them. For example, according to the report by Michalak et Al. [2] 'lifestyle habits, e.g. hair spray, hair straighteners, hair drier, drugs; dietary factors, e.g. yoghurts, lettuce, lemon, mushroom, egg, butter; other e.g. solarium, tap water pipes, tinned food, PVC foil' may probably have an influence on nickel status in the body. However, what was important to us was that we were interested to see the possible changes of this baseline (low or high), during our study period, without any major changes in lifestyle of our participants. For this reason, we used ANCOVA model for eliminating the baseline effects of ion levels.
4. The inclusion and exclusion criteria that we used were also utilized by others [2–4] and were not unusual criterion. Among the nearly 250 patients who were candidates for orthodontic appliances intervention during our sampling period, 35 eligible patients participated in this study and finally, 24 of them finished the study. The control group was selected by convenience sampling method.

5. Routinely, the authors present the results of their works via statistics. As the changes of hair nickel or chromium levels in patients who had fixed orthodontic appliances were important to us, we decided to present to the readers further data as we could. Our aim was to familiarize the readers with our data in each age and gender group. There are various reports, in smaller or bigger sample size, in which the authors disclosed their data of their participants one by one in this field or other fields [5–8] Tables 1 and 2, [6] Table 1, [7] Table 1, [8] Table 1, ... We presented the data of patient group as they were important for us and knowing the levels of metal changes for control group is unimportant. Therefore, we think this table is complete.
6. In all of the analysis, age was used as a continuous variable, not as dichotomized variable (in ANCOVA, *t*-test and the other tests). As you know, some metals are accumulated in the body over time and we were interested to examine if the levels of these ions varied in two age groups of 14–18 and 19–25 years old or not. We agree this sentence should not be inserted in the method section as we have not reported the related results.

Statistical analysis

A. We used Chi-square test for this purpose.

- Pearson correlation was used for this analysis.
- As it is mentioned in the statistical analysis and at the footnote of Table 3, independent and paired *t*-test analyses were used for these purposes.

B. We used *t*-test to compare the 'differences' between Ni and Cr levels in two genders, so we thought this sentence is correct. Perhaps, it was better to rephrase this sentence to 'there were no significant differences in scalp hair Ni and Cr levels in both genders'.

C–F. All of the inconsistencies that the letter sender pointed out were related to the Table 2. When we checked again the values of this table with our original data in our SPSS sheet, we noticed that the presented values in this table, as the letter sender concluded, are not the same as what we had in our SPSS sheet. Therefore, the figure that he had drawn and all of the analysis that he did, were on incorrect values. Unfortunately, we overlooked at this error during the writing of the manuscript and we do not know what transpired and

Corrected Table 1. Hair scalp level of nickel and chromium ($\mu\text{g/g}$ dry hair mass) in each patient at baseline and after one year.

Patient	Gender	Age	Baseline		After one year	
			Nickel	Chromium	Nickel	Chromium
1	F	14	0.0557	0.4163	0.1591	0.4574
2	F	14	0.0850	0.3115	0.1518	0.3417
3	F	14	0.0543	0.3457	0.2143	0.3537
4	M	14	0.0446	0.2290	0.1682	0.2643
5	F	14	0.0607	0.3673	0.1649	0.2952
6	M	15	0.0573	0.3686	0.2296	0.3379
7	F	15	0.0742	0.3697	0.1884	0.2118
8	F	18	0.0487	0.3319	0.1213	0.2849
9	F	15	0.0544	0.2245	0.1440	0.3241
10	M	22	0.0564	0.2711	0.1695	0.3062
11	M	17	0.0608	0.2589	0.1411	0.4511
12	F	14	0.0654	0.3854	0.2986	0.3637
13	F	14	0.1431	0.2114	0.1468	0.3223
14	F	14	0.0841	0.2426	0.1321	0.2610
15	F	15	0.0736	0.2609	0.1124	0.2839
16	F	19	0.0414	0.2544	0.1712	0.3613
17	M	15	0.0465	0.3015	0.1360	0.3263
18	F	18	0.0117	0.3111	0.1301	0.3175
19	F	14	0.0833	0.3118	0.1348	0.3403
20	M	15	0.0857	0.3047	0.0962	0.3622
21	F	14	0.0677	0.3111	0.1122	0.3980
22	F	17	0.0382	0.3401	0.0984	0.3858
23	F	14	0.0292	0.2943	0.0445	0.4874
24	M	15	0.0567	0.2800	0.1216	0.3079

missed writing the correct values. All comparisons and statistical tests that we conducted were on correct data and are correct. We apologize to the editor, reviewers, and readers of the *Acta Odontologica Scandinavica* for such an inadvertent mistake. In addition, all of the presented SEs in this table is related to triplicate test of each case (precision of the procedure) and we should not calculate the mean using these values, as the letter sender did. In other words, SEs of patient group should be calculated by considering all of the persons in this group. We provided the correct table here (Table 1, Appendix) and to prevent such incorrect calculation, only the mean of triplicate tests was presented.

G. For analyzing longitudinal data, different statistical models can be used. One of the most commonly used methods to examine the trend of change over time is the Repeated Measurement Model. In this study, at first, we used Repeated Measurement test for the assessment of interaction, but since this model is not able to eliminate the 'baseline' levels of ions, we used ANCOVA model and the results of Repeated Measurement test were not reported.

H. As the changes of hair nickel or chromium levels in patients who had fixed orthodontic appliance were important to us, we only reported the role of age and gender in this group. However, by ANCOVA model, we used all patient subjects and control group data for the analysis.

I. This was thoroughly explained in F section.

J. The means of this sentence is 'the interaction of group and gender' in statistical terminology. In the first version of the manuscript, we called this as 'Groups * Gender' that is usually utilized by a statistician [9], but according to the comments of our respected reviewers, we should change it to 'Groups adjusted with gender', and it is taken with due respect.

- 'Analysis of covariance (ANCOVA) is a technique that combines features of analysis of variance and regression. In covariance analysis terminology, each quantitative variable added to the ANOVA model is called a concomitant variable. The choice of concomitant variables is an important one. These variables are frequently used with human subjects including pre-study, age socioeconomic status and so on. A concomitant variable should be observed before the study or if it is observed during the study, it should not be influenced by the treatment in any way, ...' [9], therefore, we were able to use this test for such analysis and eliminating the 'baseline' levels of ions and our used model is correct.
- In various fields of science, the outputs are reported differently. We should report the major findings and the other findings are optional. For example, when we use *t*-test, there are many outputs, but all of them are not reported always. When we submitted the table to the journal, our respected reviewers asked us only to add the 'degrees of freedom' to the presented table.

K. We explained this in J section.

L. Due to the lack of related articles for design of control group in a before and after design at the time of designing the study, we started the study according to similar papers sample size [3, 10]. At the work ends, the power of the study was calculated, which was equal to 1.0, which means that the adequacy of the sample size was not mentioned.

Article text

1. Yes, it is right. We should rephrase that sentence and add relevant references. Thus revised form of this sentence is 'United States Environmental Protection Agency and International Atomic Energy Agency suggests that human scalp hair is exposed to many metals; however ... [4, 11].
2. Thank you very much for pointing this out. We added the relevant references to the end of this sentence. The complete form of this sentence is "...because the symptoms of a Ni allergy may be seen over time [12, 13]".
3. Although in this study, we did not have any evidence that this small change is harmful for human and may be in permissible levels, however, for hypersensitive individuals, this minimal permissible level can cause an allergic reaction [4, 8]. This sentence was the clinical significance of the study, not the study's direct findings as we stated this suggestion cautiously (by mentioning 'can be considered').
4. We did our best in terms of polishing the language of the published article and the journal expert team made some modifications on our published manuscript as well.

Disclosure statement

No potential conflict of interest was reported by the authors.

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