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LETTER TO THE EDITOR

Methodological and statistical questions regarding a study on hair nickel and chromium

Dear editors

I read with much interest the valuable study of Jamshidi et al. [1], which was an important contribution to the literature of metal ion release from orthodontic appliances and ions' systemic levels. The sample size was very large and the long duration of study made it even more valuable. The authors have collected 52 participants free of any caries and metal restoration and all patients remained this way for a whole year (I assume so, because this was an inclusion criterion). The subjects were also not exposed to nickel or chromium before the study or (supposedly) during the 1-year period of the study. That is excellent, and I am thankful to the authors for this nicely done project.

There were some questions and points and I would much appreciate if the authors could kindly address them. Perhaps these points might help in improvement of this nice report.

Methodology

1. In the methods, it is stated that 'The resulted data were reported as the means ± SE in ppb.' … Then in the Results section, the results are presented in μg/g units. To the best of my knowledge, 1 μg/g is 1 ppm and not 1 ppb. This makes me wonder if those μg/g units were correct, or if that ppb mentioned firstly was correct. So, I would appreciate any input from the authors.
2. In the methods, the authors have called this study a case-control one. They had two groups of orthodontic patients versus healthy non-patient subjects. The authors once measured all subjects' baseline ion levels and then started the orthodontic treatment in the patient group. After 1 year, they measured the ion levels of all subjects again. This design seems to be a clinical trial, in which the effect of an intervention has been tested prospectively. It does not look like a case-control study (which is a retrospective study).
3. The exclusion criteria included the exposure to nickel and chromium and having metal restorations, among others. This is very good. But baseline nickel levels of experimental/control groups had become highly significantly different (p = .000). Differences between baseline nickels of both groups implies that one of the two groups (or both of them) might have been significantly exposed to some sources of nickel; otherwise, there would be no other reason for baseline values to become different at such a high level of significance, in the absence of any interventions. There might be unaccounted sources. For example, many hair shampoos/products might contain slight traces of nickel. Such confounding factors might be the reason for the significant difference observed in baseline nickel levels. If such factors exist, the exclusion criteria were not properly controlled for some subjects.
4. The authors have mentioned very stringent exclusion criteria (no metal restorations, no exposure to nickel or chromium, and even no caries, among others). Such a design is extremely difficult to conduct and the authors are to be congratulated on this. I am interested in knowing that how many subjects were screened originally, until the authors could find 52 such purely caries-free metal-restoration-free nickel/chromium-exposure-free subjects, 24 of whom also needed bimaxillary fixed orthodontic appliances.

   Furthermore, possibly please explain how many patients were dropped out during the 1-year period of the study, due to potential caries formations or metal restorations or potential exposures to nickel or chromium during that year.

5. The raw data of patients were presented in Table 2 of Jamshidi et al [1]. But the raw data of the control group (the other 28 cases) were not presented at all. What was the reason for presenting the raw data of half of the sample (the patients), without presenting the raw data of the other half of the sample (the control group)?
6. In the methods, it is stated that 'All of the participants were in the age range of 14–25 years old and were divided into sub-groups of 14 – 18 and 19 – 25 years old.' … If the age was dichotomized into two subgroups of below 19 years and above it, how did the authors use an ANCOVA or a t-test for assessing the role of age? These tests treat age as a continuous variable, but the authors have stated that the age had been dichotomized. I checked the whole text and could not find any instances where the variable 'age' had been treated as a dichotomized variable.

Statistical analysis

1. There are tests that had not been defined in the methods section and are not known to the reader:
   - 'The difference between the gender distributions in the groups was not statistically significant (p =

.08).' No tests had been mentioned. Chi-square? Fisher? What else?
   - 'There was no significant association between the patient's age and any of the ion concentrations at any sessions ($p > .1$)'. No tests are mentioned. Please specify the test either in Results or in Methods.
   - The whole second paragraph of the Results section is full of p values, but I did not know which test had been used for computing those p values. I assumed this particular paragraph has used t-tests, but this should have been specified clearly by the authors.
2. It is stated that 'there were no significant differences between the patient's gender with any of the ion measured at any of the intervals ($p > .15$).' … Is it possible to compare gender with ion levels? I assume the word 'differences' was a mistake, and the correct word should have been 'association' or something like it. Right? If so, which test was used?
3. Inconsistency 1: it is stated in the text that the age of patients group was 15.42 ± 2.06, however, the assessment of the presented raw data of this group showed 15.54 ± 2.34.
4. Inconsistency 2: The average and SE of nickel values in baseline and after 1 year of treatment in the experimental group (reported in Table 3 of Jamshidi et al [1]) did not exactly match the average and SE calculated from the raw data disclosed in Table 2. (From raw data: baseline: 0.06542 ± 0.005077 ➞ after 1 year: 0.1490 ± 0.01024). (From Table 3 of Jamshidi et al. [1]: 0.061 ± 0.005 ➞ 0.149 ± 0.011).
5. Inconsistency 3: The average and SE of chromium levels in the experimental group (reported in Table 3 of Jamshidi et al [1]) differed considerably from the average and SE of the raw data presented as Table 2 of that paper. (From their raw data: baseline: 0.3357 ± 0.01311 ➞ after 1 year: 0.3075 ± 0.01100). (From Table 3 of Jamshidi et al [1]: 0.304 ± 0.011 ➞ 0.339 ± 0.015). This inconsistency was serious. The presented raw data shows decreased chromium levels over time, while the Table 3 and Figure 1 of Jamshidi et al. [1] both show an increase in chromium over time. Their presented raw data seemed to be inconsistent (Appendix 1). Or at least, it was not the data from which Figure 1 of Jamshidi et al [1] was created.
6. Inconsistency 4: From the presented raw data, I compared the chromium values of the patients in the baseline versus chromium levels of patients in the 1-year follow-up session. The result of paired-samples t-test was $p = .078$ (a non-significant decrease). However, Table 3 of Jamshidi et al. [1] has compared chromium levels of baseline and follow-up and has reported a significant increase ($p = .034$) for the same comparison. This inconsistency was as well serious.
7. There is this 'ANOVA repeated-measurement test' defined in the methods part of the study, but never used in the Result section. Also, it was supposed to be used for assessment of interaction, but no interactions have been analyzed in the Results section.
8. In the results section, the role of age and gender has been assessed in 'patients' only. Why it is not assessed in healthy controls but only in patients? It should have been assessed in the whole sample.
9. Inconsistency 5: It is stated that age was not associated with any ion levels in patients. I assessed the correlations between age and ion levels in patients (using the raw data presented as Table 2 of Jamshidi et al. [1]) and the association between 'age and the second nickel value' became significant. So I would appreciate if the authors could kindly explain this.
10. The results pertaining to ANCOVA (Table 4 of Jamshidi et al. [1]) look very confusing to me:
    - What does 'Groups adjusted with gender' mean? When there is already a variable called 'Groups' in Table 4, 'Groups adjusted with gender' would be incorrect.
    - Moreover, how can the (supposedly repeated-measures) ANCOVA calculate p values for some strange variable called 'baseline'? What does that even mean? The ANCOVA's p values are calculated solely for the independent variables. The baseline ion levels are not independent variables, hence calculating p values for them is technically impossible. The baseline measurements are a part of the variable 'metal ion release' which is the dependent variable. I would appreciate any explanations or corrections from the respected authors.
    - The output of SPSS's ANCOVA conveys much more needed information. Many other items of ANCOVA are missing from Table 4.
11. The description of (supposedly repeated-measures) ANCOVA in the Results section seems partially incorrect. It reads 'Results showed that by eliminating the effects of age, gender and baseline levels of Ni and Cr, … ' (Page 4). Again, the baseline levels of ions cannot have effects. The only variables that can have effects on ion levels are the independent variables. Baseline ion levels are part of the dependent variable.
12. How was the sample size determined as 24 patients and 28 controls? Why this particular number of 52 subjects?

Article text

1. In many sentences, at the end of the sentence, only one single reference is cited. For example, ' … One report by the United States Atomic Energy and Environmental Organization suggests that human scalp hair is exposed to many metals [3]' (Page 1) … In some cases, this single reference is not at all about the subject of that particular sentence. For example, the reference #3 of the article [2] has nothing to do with the report of *United States Atomic Energy and Environmental Organization*, nor has it compared mineral structure of hair with that of blood or urine.
2. There are sentences which do not cite any references, while they point to scientific facts or claims (such as this

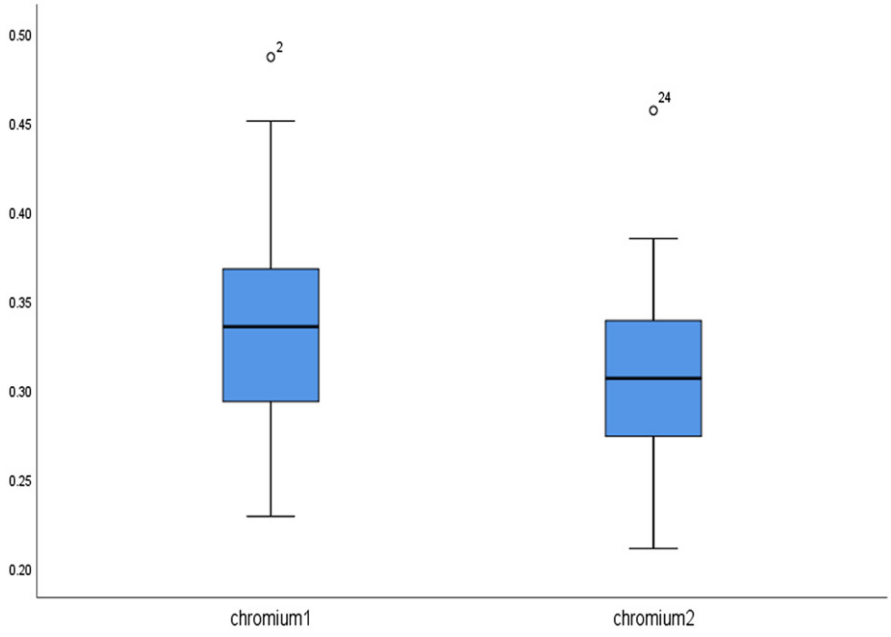
one in Page 4: 'but either way, a small level of free metal may also … be seen over time'). Such sentences need at least one relevant reference.

3. The first part of conclusions is nice and correct. However, the second part is a speculative suggestion, for which the authors have not provided any evidence. For example, on the basis of which data, the authors are certain that this level of increase in hair nickel/chromium is harmful (and therefore, 'changing the ingredients in fixed orthodontic appliances can be considered as a viable solution in the future')?
4. There were many English errors.

Disclosure statement

No potential conflict of interest was reported by the author.

Appendix



Appendix 1. Chromium levels of patients summarized by this author from the raw data presented in Table 2 of jamshidi et al[1]

References

[1] Jamshidi S, Rahmati Kamel M, Mirzaie M, et al. Evaluation of scalp hair nickel and chromium level changes in patients with fixed orthodontic appliance: a one-year follow-up study. Acta Odontol Scand. 2018;76:1–5. doi:0.1080/00016357.2017.1372624.

[2] Amini F, Mollaei M, Harandi S, et al. Effects of fixed orthodontic treatment on hair nickel and chromium levels: a 6-month prospective preliminary study. Biol Trace Elem Res. 2015;164:12–17.

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