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The Effect of the Water Boiled in Aluminum Cookware on Chromatid Breaks in Human Blood Lymphocytes

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Dear Editor,

Aluminum (Al) can occur in foods as a result of using Al containing food additives and cooking and packaging of foodstuffs in the Al utensils and foils.[1] This metal has been suggested to affect the central nervous system and contribute to neurodegenerative abnormalities as well as osteomalacia and microcytic anemia.[2] Previously, we showed a marginal significant increase in the prevalence of total genomic abnormalities in the meristematic cells of onion root from the groups treated with the water boiled in Al pots compared to the control.[3] In the present study, we planned to examine the genotoxic effect of the water boiled in an Al cookware on chromatid breaks in cultured human lymphocytes. For this aim, distilled water was boiled in a used Al cookware with high flames for more than 20 hours, and then the concentration of Al was measured by atomic absorption spectrometer. Samples from the peripheral blood of 4 unrelated healthy females aged 19-45 years were inoculated in RPMI 1640 medium enriched with PHA, FBS, penicillin-streptomycin, and L-glutamine and then incubated at 37°C under 5% CO_2. After 24 hours, the cultured cells were exposed to the water boiled in the Al cookware with Al concentrations of 0.9, 4, and 8 mg/L and incubated for 48 hours. For each individual, untreted cultured lymphocytes were used as a negative controle. For positive control, 0.4 µg/mL of mitomycin C was applied with a blood sample from a healthy female in three replicates. To stop the cells at the metaphase, 45 min before harvesting, 300 µM of colcemid was added to the cultures. At the end of the incubation time, the prepared slides from Giemsa stained cells were analysed for chromatid breaks in 90-100 metaphases for each individual at each Al concentration and negative control using light microscopy. It should be mentioned that for three replicats of the positive control, we could get just 16, 19, and 74 metaphases. Student's *t* test and one-way ANOVA were performed to analyze the data using SPSS version 22, and significance was accepted at $P<0.05$.

The data of chromatid breaks have been demonstrated in Table 1. The result of the Student's *t* test indicated that there was a significant increase in the percentage of chromatid breaks in the positive control compared to the negative control (t=-3.40, $P=0.019$). However, ANOVA analysis showed that there was no significant difference in the frequency of chromatid breaks between the Al-treated groups and the negative control group (F=0.218, $P=0.882$). Using the comet assay, previous studies reported that the level of DNA damage increased in the human cultured lymphocytes exposed to $AlCl_3$ as well as in the lymphocytes of the carps living in an environment containing Al.[4,5] Our data; however, did not confirm the adverse effect of the water boiled in the Al cookware on the chromosomes of human lymphocytes. To our knowledge, this is the first preliminary study to examine the genotoxic effect of the water boiled in an Al cookware on human lymphocytes. Further research with more sophisticated methods and larger samples of pots is required to confirm the safety of these cookwares.

Ethical Approval

Not applicable.

Competing Interests

The authors have no conflict of interest to declare.

Authors' Contribution

SNSH carried out the experiment, statistical analyses, and drafting of the manuscript. ZZ designed the study participated in revising the manuscript and approving the final manuscript.

Table 1. Chromatid Breaks in the Lymphocytes Treated with Water Boiled in an Aluminum Cookware

Treatment Groups Al Concentration (mg/L)	Chromatid Break Percent (Mean ± SE)
0.9	1.29 ± 0.99
4	1.00 ± 0.41
8	1.76 ± 0.24
Negative control	1.54 ± 0.86
Positive control	11.33 ± 3.22

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