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Oxidative Stress, DNA Damage and Apoptosis Levels in Those Who Use Borderline High Level Fluoride Content Drinking Water

Sınırda Yüksek Düzeyde Florid İçeren İçme Suyu Kullananlarda Oksidatif Stres, DNA Hasarı ve Apoptoz Düzeyleri

Adnan Kirmit[1], Mehmet İrfan Yeşilnacar[2], Metin Çalışır[3], İbrahim Bayhan[4], Hakim Çelik[5]

[1] Department Of Biochemistry, Faculty Of Medicine, Harran University, Sanliurfa, Turkey·
[2] Department Of Environmental Engineering, Engineering Faculty, Harran University, Şanlıurfa, Turkey,
[3] Department Of Periodontology, School Of Dentistry, Adıyaman University, Adıyaman, Turkey·
[4] Public Health Directorate, Environmental Health Branch, Sanliurfa, Turkey·
[5] Department Of Physiology, Faculty Of Medicine, Harran University, Sanliurfa, Turkey·

Abstract

Aim: Fluoride is necessary for tooth and bone development, but when exposed to excessive levels can cause oxidative stress, DNA damage, apoptosis, fluorosis and cancer. The aim of this study is to reveal the underlying mechanism of fluoride toxicity and to clarify, in part, the uncertainty of the fluoride level in the reference value ranges of drinking water.

Material and Method: Two groups were included in the study as exposure and control groups. Serum Total Oxidant Status and Total Antioxidant Status were measured with colorimetric; Urine 8-OHdG (8-hydroxy-2-deoxyguanosine) levels as DNA damage biomarkers and serum M30 and M65 levels as apoptosis biomarkers were studied by ELISA method. In addition, all participants underwent a dental examination by the dentist.

Results: Serum total antioxidant status (TAS) were lower in the exposure groupcompared to the control group($p<0.001$); serum total oxidant status (TOS) ($p<0.001$), OSI ($p<0.001$), M30 ($p<0.001$) and M65 ($p<0.001$) levels and urine 8-OHdG ($p=0.011$) levels were high. However, the M30 / M60 ratio was not statistically different between the two groups ($p=0.371$). Dental fluorosis was detected in all participants in the exposure group.

Conclusion: This study showed increased levels of oxidative stress, DNA damage and apoptosis biomarkers in drinking water users with borderline high level fluoride. Therefore, instead of the World Health Organization's reference value range (0.5-1.5 mg/L) for fluoride levels in drinking water, the US Public Health Service's (0.7 mg/L) reference value range seems to be more appropriate to the precaution.

Keywords: Fluoride, Oxidative stress, DNA damage, 8-OHdG, Apoptosis, M30, M65, Fluorosis.

Öz

Amaç: Florid, diş ve kemik gelişimi için gereklidir, ancak aşırı seviyelerde maruz kaldığında oksidatif stres, DNA hasarı, apoptoz, floroz ve kansere neden olabilmektedir. Bu çalışmanın amacı, florid toksisitesinin altta yatan mekanizmasını ortaya çıkarmak ve kısmen, içme suyu florid seviyesinin referans değer aralıkları arasındaki belirsizliğini açıklığa kavuşturmaktır.

Gereç ve Yöntemler: Çalışmaya maruziyet ve kontrol grubu olmak üzere iki grupdahil edildi. Serum Toplam Oksidan Seviyesi ve Toplam Antioksidan Seviyeleri kolorimetrik; DNA hasarı biyobelirteci olarak idrar 8-OHdG (8-hidroksi-2-deoksiguanozin) seviyeleri ve apoptoz biyobelirteci olarak serum M30 ve M65 seviyeleri ELISA yöntemi ile çalışılmıştır. Ayrıca, tüm katılımcılara diş hekimi tarafından diş muayenesi yapıldı.

Bulgular: Serum total antioksidan seviye (TAS) maruziyet grubunda kontrol grubuna kıyasla daha düşüktü ($p<0,001$); serum total oksidan seviye (TOS) ($p<0,001$), OSI ($p<0,001$), M30 ($p<0,001$) ve M65 ($p<0,001$) seviyeleri ve idrar 8-OHdG ($p=0,011$) seviyeleri yüksekti. Ancak, M30 / M60 oranı iki gruparasında istatistiksel olarak farklı değildi ($p=0,371$). Maruziyet grubundaki tüm katılımcılarda dental florozis saptandı.

Sonuç: Bu çalışma, sınırda yüksek düzeyde florid içeren içme suyu kullananlarda oksidatif stres, DNA hasarı ve apoptoz biyobelirteçlerinin arttığını göstermiştir. Bu nedenle, Dünya Sağlık Örgütü'nün içme sularında florid seviyeleri için referans değer aralığı (0,5-1,5 mg/L) yerine ABD Halk Sağlığı Hizmetleri biriminin (0,7 mg/L) referans değer aralıkları ihtiyata daha uygun görünmektedir.

Anahtar Kelimeler: Florid, Oksidatif stres, DNA hasarı, 8-OHdG, Apoptoz, M30, M65, Florozis.

Corresponding *(İletişim)*: Adnan Kirmit , Dr. Öğr. Üyesi (Tıp Doktoru). Harran Üniversitesi Tıp Fakültesi Araştırma ve Uygulama Hastanesi, Biyokimya Laboratu- varı. Osmanbey kampüsü, 63290, Haliliye/Şanlıurfa,Turkiye
E-mail *(E-posta)*: adnankirmit@yahoo.com
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INTRODUCTION

One of the inorganic substances that the organism needs and needs to be taken from outside is fluoride.[1,2] Although fluoride is one of the vital elements for human health, it has a doubleedged sword effect on human health.[3] When taken at the normal dose, it contributes to bone and tooth development, and when taken at an excessive level, it can cause dental, skeletal and visceral fluorosis and cancer depending on the dose and duration of exposure.[4,5] Fluoride is the ion form of fluorine, which is gas and neutral, and is found in compounds, not free in nature. It is mostly found as Sodium Fluoride (NaF_2) and Calcium fluoride (CaF_2). Fluoride is found in the atmosphere, soil, water and food.[6] Surface waters generally do not contain more than 0.3 mg/L of fluoride unless they are contaminated from external sources. However, depending on many variables, fluoride concentrations in groundwater have a wide range (1.0 35.0 mg/L). The main source of people's daily fluoride consumption is drinking water (75-90% of daily intake).[7]

Prolonged consumption of drinking water containing fluoride ions greater than 1.5 mg/L causes fluorosis in the teeth and skeletal system, neurological damage and further toxic effects are seen when exposed to concentrations of more than 4 mg/L.[8] Previous studies have showed that fluorine can induce genotoxicity, cytotoxicity, immunotoxicity, oxidative damage, apoptosis and lesions in the broiler peripheral blood, liver, kidney, thymus, spleen, cecal tonsil, and intestine, and in the mouse spleen.[9-16]

Reactive oxygen species (ROS), one of the by-products of the metabolic process, can normally be removed by numerous antioxidant defence components.[17] The imbalance between ROS and antioxidants is called "oxidative stress".[18] Fluoride is known to be an inhibitor of antioxidant enzymes, which promotes ROS accumulation.[19,20]

Due to the acceptance of fluoride as an environmental pollutant, many studies have been conducted on exposure to fluoride. In the studies, it was found that exposure to fluoride could induce oxidative stress in liver, kidneys, testis, spleen, brain, heart and cecal tonsils and reduce the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-PX) and glutathione-s-transferase (GST).[21-26]

The most important free oxygen radical that damages cellular biomolecules (proteins, membrane lipids and DNA) is the hydroxyl radical (HO). 8-OHdG (8-hydroxy-2-deoxyguanosine) is one of the main biomarkers of damage caused by free radicals in nuclear and mitochondrial DNA and is widely used for this purpose.[27] Cells whose DNA is irreparably damaged must be removed by apoptosis as they may have the potential for malignancy. Lack of apoptosis can cause cancer and autoimmune diseases.[28]

In cell death, creatine (CK18) is released outside the cell. The M30 measurement kit detects a neoepitope of a fragment of CK18 that appears only after caspase cleavage with positions 387 to 396, and is considered a selective biomarker of apoptosis.[29]

The M65 kit detects a common epitope found in both unfragmented CK18 (released from necrosis cells) and its fragment (released from apoptotic cells) and is therefore considered a marker of total cell death (apoptosis and necrosis).[30] Therefore, high M30/M65 ratio shows that cell death is mainly due to apoptosis.[31,32] The increase in isolated M65 indicates necrosis.[33]

As the concentration of toxic substance increases, apoptosis increases up to a point, then apoptosis decreases and the increases in toxic substance concentration, resulting in a linear increase in necrosis.[31]

The World Health Organization (WHO) states that the reference range for fluoride in drinking water is 0.5–1.5 mg/L.[34] The American Environmental Protection Association (EPA) and the American Public Health Service (PHS) previously reported the fluoride level in drinking water to be 0.7–1.2 mg/L, while the PHS revised its optimal value to 0.7 mg/L.[35] EPA stated that there is no need for a reference update for the time being but it will continue to follow up the researches done on this subject and go to the reference regulation when necessary.[36]

Although there are many studies in the literature regarding the outcome of the fluoride effect, most of these are limited to animal experiments using drinking water containing very high amounts of fluoride. There is still uncertainty as to whether a high level of fluoride at the boundary in drinking water is toxic. This prospective study was designed to investigate whether the use of drinking water with borderline high level fluoride is dangerous or not.

MATERIAL AND METHOD

Research Population

Two groups, including exposure and control group, were included in the study. The exposure group consisted of 39 participants, 20 males and 19 females between 7-14 years of age who used drinking water with borderline high level fluoride (1.7±0.27 mg/L). The control group consisted of 39 participants that statistically similar age and gender and use containing normal fluoride (0.9±0.18 mg/L) drinking water. Before sampling, the families were informed about the study and after the consenting parents signed the informed consent form, demographic characteristics of the participants were recorded and blood and urine samples were taken.

In our study Dean's index.[37]that recommended by WHO to diagnose dental fluorosis was adopted. The same periodontist was specified to conduct all the testing measurements to guarantee the accuracy and consistency for diagnostic criteria.

Sampling

Blood samples taken from the antecubital region of the participants in the sitting position after the rest, were centrifuged at 3000 rpm for 10 minutes after clotting and stored at -80 °C. After the urine samples were taken into a sterile container and centrifuged, the supernatant was stored at -80 °C. On the working day, all samples were allowed to dissolve at room temperature and then the tests were performed.

Biochemical Analyses

Serum M30 and M65 levels (PEVIVA, Bromma, Sweden) and urine 8-OHdG levels were studied by ELISA (CUSABIO, Wuhan, China) and TAS and TOS were determined by Erel's colorimetric method.[38] Fluoride levels in drinking water were measured by portable Hach-Lange HQ40d Multi meter and TISAB method. The fluoride levels were analysed from the samples taken four times a month.

Statistical Analyses

The data obtained from the study were performed with SPSS 22.0 Package Program (Inc, Chicago, IL, USA). Normal distribution tests of continuous variables were performed by Kolomogorov-Smirnov test. As the results of the analysis showed that the parameters had normal distribution, the mean differences between the groups were compared with Independent Samples t Test. The results were evaluated at 95% ($p<0.05$) significance level.

RESULTS

Body Mass Index (BMI) and mean age of the exposure and control groups included in the study were statistically similar ($p>0.05$) (**Table 1**).

Table 1. Comparison of gender, age and BMI values of the groups

	Control Group (n:39)	Exposure Group (n:39)	p
Gender (F/M) χ	19/20	19/20	1
Age (Year)#	10.41±2.27	10.62±2.25	0.691
BMI (kg/m2)#	17.46±0.92	17.28±0.87	0.381

#: Independent Samples t Test; Mean±SD, χ: Chi-square test,

Dental fluorosis was detected in all participants in the exposure group. Serum TAS were significantly lower and TOS and OSI were higher in the exposure group compared to the control group ($p<0.001$) (**Table 2**).

Urine 8-OHdG levels were significantly higher in the exposure group compared to the control group ($p<0.05$) (**Table 2**). Although the mean M30 and M65 were significantly higher in the exposure group compared to the control group ($p<0.001$), the M30/M65 ratio was lower in the exposure group and the difference between the groups was not statistically significant ($p>0.05$) (**Table 2**)

Table 2. Comparison of serum parameters of group's#

	Control Group (n:39)	Exposure Group (n:39)	p
8-OHdG (ng/mL)	7.31±3.42	9.59±4.25	0.011
TAS (mmol trolox eqv/L)	1.83±0.32	1.28±0.20	<0.001
TOS (μmol H2O2 eqv/L)	0.99±0.24	1.68±0.47	<0.001
OSI (AU)	0.54±0.15	1.32±0.47	<0.001
M30 (U/L)	201.24±71.54	272.16±84.85	<0.001
M65 (U/L)	472.16±168.60	671.86±216.12	<0.001
M30/M65 (AU)	0.472±0.197	0.435±0.165	0.371

#: Independent Samples t Test, Mean±SD,

DISCUSSION

Reactive oxygen species (ROS) have been shown as pathological mediators for most diseases.[39] Several studies have been conducted on the relationship between high levels of fluoride exposure and free radical

formation.[40 41] In some studies, it has been shown that excessive fluoride level can induce lipid peroxidation. [41] However, it has been reported that antioxidant enzyme activities in kidneys and liver are decreased and antioxidant content decreases due to high levels of fluoride exposure in animals and this causes excessive free radical accumulation.[40] While some authors state that fluoride exposure does not increase free radical formation, some authors state that NaF causes apoptosis, but it does this without increasing ROS production.[42,43] Some authors state that low concentrations of fluoride increase cell proliferation, and in high concentrations, it decreases and induces apoptosis.[4446] All these results show that free radicals play an important role in the pathogenesis of fluorosis.

As a result of the statistical analysis serum TAS were significantly lower and TOS and OSI values were higher in the exposure group compared to the control group ($p<0.001$). In many studies, it has been reported that fluoride induces lipid peroxidation, increases free radical production and decreases TAS.[47,48]

Urine 8-OHdG levels were significantly higher in the exposure group compared to the control group. In a study investigating the protective effect of resveratrol against oxidative stress caused by sodium fluoride, a decrease in TAS and an increase in TOS and 8OHdG levels were observed in the fluoride exposure group compared to the control group.[49]

Although the mean M30 and M65 levels were significantly higher in the exposure group compared to the control group, the M30/M65 ratio was lower in the exposure group and the difference between the groups was not statistically significant. Cytokeratin 18 (CK18) is an epithelial cell-specific intermediate filament released into the circulation during cell death.[50]

There were some limitations in our study. First of all, this study was cross-sectional and the diets of both groups were not controlled. Secondly, it would be more appropriate if we did this study in a residential area using drinking water containing fluoride at the level of 1.2-1.5 mg/L, which is actually between the WHO and Institute of Turkish Standards (TSE) and the reference value ranges determined by EPA for the fluoride level in drinking water. However, since the drinking water containing fluoride at the level closest to this controversial range is in this field, we conducted our study in this field.

CONCLUSION

Asaresult, this study showed increased levels of oxidative stress, DNA damage and apoptosis biomarkers, even at borderline high levels of fluoride exposure, according to WHO's reference range for fluoride in drinking water. This can be interpreted as the fact that fluoride is genotoxic, even at borderline high levels. In addition, dental fluorosis was observed even at the borderline high level of fluoride exposure. In this regard, the reference value ranges determined by EPA (0.7-1.2 mg/L) or PHS (0.7 mg/L) for fluoride levels in drinking water seems to be more prudent than those of WHO (0.5-1.5 mg/L).

ETHICAL DECLARATIONS

Ethics Comittee Approval: For this study, permission was obtained from Harran University Faculty of Medicine Ethics Committee with the decision numbered 26 of the session numbered 4, dated 06.04.2017.

Informed Consent: Written informed consent was obtained from all participants who participated in this study.

Status of Peer-review: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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