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Relationship Between Plasma Fluoride Levels, Glutathione Peroxidase Activity, Hemoglobin, and Abortion in Rural and Urban Pregnant Women from Settat (Morocco)

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Abstract

Background: This study aimed to investigate the relationship between plasma fluoride levels, glutathione peroxidase activity, hemoglobin, and abortion among rural and urban pregnant women from Settat province (Morocco).

Methods: Blood samples were collected from rural pregnant women who had not undergone abortion (N = 224), rural pregnant women who had an abortion (N = 38), urban pregnant women who had not undergone abortion (N = 163), and urban pregnant women who had an abortion (N = 14).

Results: The highest ($p < 0.01$) plasma fluoride levels and the lowest ($p < 0.01$) GPx activity were observed in rural pregnant women. In all participants, plasma fluoride levels were significantly higher ($p < 0.001$), and the GPx activity was significantly ($p < 0.001$) lower in pregnant women who had an abortion compared with those who did not undergo such process. No significant difference was observed between the hemoglobin levels of all participants. Furthermore, abortion was positively correlated with plasma fluoride levels ($p < 0.001$) and negatively correlated with GPx activity ($p < 0.001$) in rural participants.

Conclusions: Rural pregnant women had higher plasma fluoride levels and lower GPx activity, which correlated with the increased abortion risk and oxidative stress.

Keywords: abortion, glutathione peroxidase, hemoglobin, plasma fluoride, pregnancy, women

INTRODUCTION

Fluoride belongs to the halogen family. This element is widely distributed in the environment, mainly in soil, air, and water.[1] At recommended doses, fluoride plays an essential role in increasing the structural stability of teeth and bones. It is also involved in human and animal growth.[2] However, chronic exposure to high fluoride levels can lead to chronic intoxications, such as dental and skeletal fluorosis.[3] Furthermore, prolonged exposure to this halogen can induce other toxic effects in the reproductive, nervous, and immune systems, which lead to nonskeletal fluorosis.[4] Fluorosis is an important public health concern in many parts of the world.[5] Moreover, fluoride intake through groundwater is a major contributor to this problem and has become one of the most critical issues affecting human health.[6] Normally, drinking water is considered contaminated when its fluoride level is between 1.1 and 2.5 ppm and toxic at fluoride levels greater than 2.5 ppm.[4] High fluoride levels in well water have been reported in several Moroccan endemic fluorosis areas.[7,8] For instance, in a study on the Bni Meskine community in the province of Settat, Morocco, the fluoride concentration was higher than the permissible limit for drinking water standards.[9]

Previous studies have emphasized that after its absorption, fluoride can bind divalent elements, such as selenium.[10] The latter is essential for the activity of glutathione peroxidase (GPx), an enzyme involved in stress response and antioxidant maintenance.[11] This bond between fluoride and selenium suggests that fluoride excess can indirectly inhibit GPx activity by binding to selenium, which results in the accumulation of reactive oxygen species (ROS) and oxidative stress.[12] Oxidative stress has been increasingly reported to cause several pregnancy complications, such as abortion and preeclampsia.[13] Oxidative stress can also result in the destruction of red blood cells through hemolysis.[14] Recently, epidemiological studies in some endemic fluorosis areas have shown the relationship between fluoride levels and pregnancy complications among women.[15–17] However, these research encountered methodological limitations, given that they only focused

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on the correlation between plasma or urinary fluoride and pregnancy complications. Therefore, other biomarkers must be investigated to understand further the possible mechanisms by which fluoride can lead to pregnancy complications. Fluorosis is endemic in Settat province,[18,19] which is composed of rural areas, where water is mainly wells, and urban areas, where water is tap water, which is filtered and controlled. This condition suggests the higher exposure of rural populations to fluoride excess. Based on the data mentioned above, the current study aimed to investigate the relationship between plasma fluoride levels, glutathione peroxidase activity, hemoglobin, and abortion in rural and urban pregnant women from Settat province (Morocco).

METHODS

This research was conducted per the principles of the Helsinki Declaration of the World Medical Association in 1964 and the Moroccan Ministry of Health recommendations relating to interventional biomedical research. To ensure ethical compliance, we submitted a formal request for approval through established hierarchical channels. To do so, we sought endorsement from the Hassan II Hospital management in Settat, the Provincial Health Delegation in Settat, the Regional Health Management of the Casablanca Settat region, and the Ministry of Health of Morocco, specifically the Directorate of Epidemiology and Disease Control. We successfully obtained Ethical Approval No. 1295/18 from the Ministry of Health of Morocco after the validation of our study.

This study was conducted on pregnant women from Settat province, Morocco, who visited the provincial public hospital Hassan II for an assessment of their pregnancy or consultation regarding an abortion. The study was conducted between June and December 2019. A total of 439 participants, aged 20–35 years old and residents of Settat province, were included in this research. All ethical principles, including free choice, informed consent, respect for confidentiality, and anonymity, were considered throughout the research. Of the 262 pregnant women from rural areas, 38 reported having undergone an abortion. Meanwhile, among 177 pregnant women from urban areas, 14 had an abortion. All participants aged less than 20 years, over 35 years, who required an emergency intervention, or who had other high-risk pregnancies were excluded. A convenience sampling technique was employed in this study. Pregnant women who visited the provincial public hospital Hassan II during the specified period were invited to participate based on their availability and willingness to participate.

Samples were collected from venous blood using heparin and ethylenediaminetetraacetic acid tubes to measure plasma fluoride levels and GPx activity, respectively. The samples were centrifuged at 3000 g for 15 min to separate the plasma and erythrocytes.

One volume of total ionic strength adjustment buffer was added to the same volumes of samples. Plasma fluoride levels, which were expressed as mg/l, were measured using a fluoride electrode (Thermo Scientific Orion 96-09, Orion Research, Cambridge, MA, USA) coupled to an analyzer ion (Star A214, Thermo Scientific Orion). The electrode was calibrated with standard fluoride solutions at concentrations of 0.025, 0.050, 0.075, and 0.1 mg/l and prepared with the same reagent used for the samples.

The GPx activity was measured following the method of Flohé and Günzler.[20] Briefly, the sample (60 µl) was incubated for 15 min at 37 °C in a reaction mixture containing 60 µl potassium phosphate buffer (0.1 M, pH 7.0), 40 µl reduced glutathione (GSH; 2 mM), 20 µl hydrogen peroxide (H_2O_2; 1 mM), and 20 µl sodium azide (1 mM). Then, 100 µl trichloroacetic acid (5%) was added to stop the reaction. After centrifugation for 5 min at 1500 g, 20 µl supernatant was collected and added to 40 µl phosphate buffer (50 mM, pH 7.0) and 140 µl 5,5-dithiobis (2-nitrobenzoic acid (0.4 mg/ml). The absorbance was read at 420 nm using a microplate reader (2100-C, Optic Ivymen Systems™, COMECTA®). The GPx activity was expressed as micromoles of GSH per minute per milligram of protein (U/mg of proteins).

The protein content in erythrocytes was estimated in accordance with the procedure described by Lowry *et al*.[21], with bovine serum albumin used as a standard. Meanwhile, hemoglobin levels were determined using an automatic hematology analyzer H 360 and expressed as g/dl.

Statistical analyses were performed using the JMP11.0 software (SAS Institute Inc., Cory, NC, USA). The Kolmogorov–Smirnov test was used to evaluate whether the data were normally distributed. The non-normally distributed numeric data were compared using the Mann–Whitney U test.[22] The correlation between abortion, plasma fluoride, GPx, and hemoglobin in rural and urban pregnant women was analyzed using Spearman's Rho test.[23]

RESULTS

Table 1 summarizes the plasma fluoride levels, GPx activity, and hemoglobin levels in rural and urban pregnant women. The results revealed that the average plasma fluoride levels were significantly higher ($p < 0.01$) in rural pregnant women (0.037 ± 0.008) than in urban pregnant women (0.035 ± 0.007). Moreover, the average GPx activity was significantly lower ($p < 0.01$) in rural pregnant women (68.10 ± 7.87 U/mg proteins) than in urban pregnant women (70.88 ± 7.78 U/mg proteins). No significant difference was observed in the hemoglobin levels.

The results in Table 2 show that the average plasma fluoride levels were significantly higher ($p < 0.001$) in

pregnant women who had an abortion (rural: 0.043 ± 0.009 mg/l; urban: 0.038 ± 0.006 mg/l) compared with those who had not (rural: 0.036 ± 0.007 mg/l; urban: 0.034 ± 0.007 mg/l). In addition, the average plasma fluoride levels were significantly higher ($p < 0.001$) in rural pregnant women who had and had not undergone abortion compared with their urban pregnant counterparts (Table 2).

The results in Table 2 also indicate that the average GPx activity was significantly lower ($p < 0.001$) in pregnant women who had an abortion (rural: 57.07 ± 1.02 U/mg proteins; urban: 57.85 ± 0.93 U/mg proteins) compared with those who had not undergone such process (rural: 69.97 ± 6.93 U/mg proteins; urban: 72.01 ± 7.06 U/mg proteins). Furthermore, the average GPx activity was significantly lower ($p < 0.001$) in rural pregnant women who had and who did not have an abortion compared with their urban counterparts (Table 2). As shown in Table 2, no significant difference was observed in hemoglobin levels between all participants.

The results of the Rho Spearman test (Table 3) revealed that in rural pregnant women, abortion was positively correlated with plasma fluoride levels (r = 0.2564; $p < 0.001$) and negatively correlated with the GPx activity (r = −0.520; $p < 0.001$). Furthermore, a negative correlation was observed between plasma fluoride levels and GPx activity (r = −0.2757; $p < 0.001$). For the urban pregnant women, the results indicated that abortion was negatively correlated with the GPx activity (r = −0.4917; $p < 0.001$). Moreover, a negative correlation was observed between fluoride and GPx activity (r = −0.1859; $p < 0.01$).

TABLE 1. Plasma fluoride, GPx activities, and hemoglobin levels in rural and urban pregnant women

Origin	Rural	Urban	*p*
Plasma fluoride (mg/l)	0.037 ± 0.008	0.035 ± 0.007	0.004
GPx (U/mg of proteins)	68.10 ± 7.870	70.88 ± 7.780	0.001
Hemoglobin (g/dl)	11.19 ± 1.250	11.17 ± 1.280	0.772

The results are expressed as average ± standard deviation

TABLE 2. Fluoride levels, GPx activities, and hemoglobin levels in rural and urban pregnant women who had or did not have an abortion

Origin	Rural	Urban	*p*
Plasma fluoride (mg/l)			
Without abortion	0.036 ± 0.007	0.034 ± 0.007	0.028
With abortion	0.043 ± 0.009	0.038 ± 0.006	0.086
p	0.001	0.007	
GPx (U/mg of proteins)			
Without abortion	69.97 ± 6.93	72.01 ± 7.06	0.003
With abortion	57.07 ± 1.02	57.85 ± 0.93	0.004
p	0.001	0.001	
Hemoglobin (g/dl)			
Without abortion	11.22 ± 1.25	11.18 ± 1.29	0.775
With abortion	11.03 ± 1.26	11.02 ± 1.16	0.620
p	0.215	0.339	

The results are expressed as average ± standard deviation

TABLE 3. Correlation between abortion, fluoride levels, GPx activity, and hemoglobin levels in rural and urban women

Residential area	Abortion	Fluoride	GPx	Hemoglobin
Rural				
Abortion	1.0000**	0.2564**	−0.5780**	−0.0520
Fluoride	0.2564**	1.0000**	−0.2757**	−0.1068
GPx	−0.5780**	−0.2757**	1.0000**	0.0516
Hemoglobin	−0.0520	−0.1068	0.0516	1.0000**
Urban				
Abortion	1.0000**	0.1372	−0.4917**	−0.0350
Fluoride	0.1372	1.0000**	−0.1859*	−0.0094
GPx	−0.4917**	−0.1859*	1.0000**	0.0146
Hemoglobin	−0.0350	−0.0094	0.0146	1.0000**

**Correlation is significant at the 0.001 level; * Correlation is significant at the 0.01 level

DISCUSSION

In Moroccan endemic areas, such as El Brouj, Beni Meskin, and Ben Hmed, which are of the Settat province, fluoride is naturally present in phosphate rocks; hence, weathering in these locations releases a high amount of fluoride that contaminates the groundwater.[4] Moreover, a study in this province revealed a high fluoride concentration in groundwater,[9] whose daily consumption can lead to diverse harmful effects. Given that blood is the main transporter of fluoride in the body,[24] plasma fluoride level is an essential parameter to diagnose the chronic toxic effects of fluoride, mainly in pregnant women.[15] The results of the current study revealed significantly higher average plasma fluoride levels in rural pregnant women than those in urban areas. The drinking water sources in each area can explain this finding. Specifically, the rural population consumes well water contaminated by excess fluoride in phosphate rocks, whereas the urban population uses tap water, which is filtered and controlled. Similarly, a study conducted in India revealed a positive correlation between plasma fluoride in pregnant women and fluoride in groundwater.[16]

Comparison of the values obtained in this previous study revealed blood fluoride levels lower than those obtained in pregnant Polish women[17] and higher than those recorded by other research.[15,25] Fluoride is transported across the placenta during pregnancy[6,26]; exposure to a high fluoride level during this critical physiological stage can cause abortion,[27] congenital abnormalities, intrauterine fetal death, anemia, neurotoxicity of the fetal brain, low birth weight, preterm delivery, and poor Appearance, Pulse, Grimace response, Activity, and Respiration (APGAR) score.[6,28–30] Therefore, the relationship between plasma fluoride levels and abortion in pregnant women was investigated in the present research. The results showed that in rural and urban areas, the plasma fluoride levels were significantly higher in pregnant women who had an abortion than those who did undergo the process, which agrees with the aforementioned studies' findings. On the other hand, previous studies reported that some factors, such as malnutrition,[31] infections,[32] and environmental pollutants,[33] can cause oxidative stress, which leads to the progression of several pregnancy complications, such as abortion, preterm birth, miscarriages, gestational diabetes, and fetal growth restriction.[34–36] However, to our knowledge, none of such studies have documented the associations regarding fluoride, antioxidant status, and adverse pregnancy outcomes. Therefore, in the present work, the relationship between plasma fluoride levels, GPx activity, and abortion was investigated. Our findings revealed that erythrocyte GPx activity was significantly lower in pregnant women who had an abortion than those who had not. Hence, from these results, fluoride can increase the risk of abortion in pregnant women via oxidative stress pathways.

As highlighted previously, fluoride is an electronegative element, and after its absorption, it binds to several divalent elements, such as calcium, magnesium, zinc, and selenium.[11] Selenium is a necessary cofactor for the activity of the selenoenzyme GPx, which is involved in the stress response and maintenance of high levels of antioxidants in the body.[12] Hence, excess fluoride can inhibit the GPx activity by binding to selenium, which leads to ROS accumulation and oxidative stress. Superoxide dismutase and GPx are the main antioxidant enzymes;[37] the first catalyzes the dismutation of superoxide anion free radical (O_2^-) into molecular oxygen and hydrogen H_2O_2,[38] and the second catalyzes the transformation of H_2O_2 into H_2O. In this study, the negative correlation between plasma fluoride levels and the GPx activity was possibly due to the inhibition of GPx by fluoride, which can pave the way for Fenton's reaction, which decomposes hydrogen peroxide catalytically to generate hydroxide (OH^-) and hydroxyl radicals ($HO^•$). Furthermore, these powerful oxidizing agents cause oxidative stress,[39] which can result in aberrant spiral artery formation, increased placental vascular resistance, and decreased uterine perfusion. In addition, ROS can damage DNA, lipids, and proteins, shorten telomeres, accelerate the aging of fetal membranes, and induce the aging of placental structures, which leads to their failure and the development of pregnancy complications such as abortion.[40] ROS can also damage endothelial cells, resulting in an altered prostacyclin–thromboxane balance that leads to preeclampsia or abortion.[13]

This study is the first of its kind in Morocco, and it focuses on abortion and its relationship with fluoride exposure. However, this research encountered several limitations. Most importantly, obtaining a sufficiently large sample size of pregnant women meeting the specified characteristics was challenging. Moreover, some participants did not provide the necessary information to determine whether their abortion was related to fluoride exposure or other causes.

CONCLUSIONS

In conclusion, rural pregnant women had higher plasma fluoride levels and lower GPx activity than those in urban areas. Abortion was positively correlated with plasma fluoride levels and negatively correlated with the GPx activity in rural pregnant women. These findings suggest a potential link between excessive fluoride exposure and increased abortion risk, possibly mediated by oxidative stress. Extensive biochemical and molecular studies are needed to further gain insights into the underlying mechanisms behind abortion in areas with endemic fluorosis. Future research will focus on the specific biochemical and molecular pathways involved in the association between fluoride excess and abortion to

develop targeted prevention measures to mitigate the associated risk.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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