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Modification of the Genetic Polymorphism of Glutathione-S-Transferase (GSTM1 and GSTT1) in Motorcycle Drivers Exposed to BTEX in Cotonou

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Abstract

The glutathione-S-transferase genes mainly the GSTM1 and GSTT1 alleles are responsible for the synthesis of detoxication enzymes that can remove toxic substances. The objective of this study is to seek changes in the genetic polymorphism of glutathione-S-transferase GSTM1 and GSTT1 in motorcycle drivers exposed to BTEX. Our study group consists of 60 motorcycle drivers including 30 professional and 30 non-professional. Blood samples were preleveled from the study population in the EDTA tubes and DNA was extracted by the phenol/chloroform method. The PCR technique was used to determine the presence or absence of genes. Our results showed that the percentage of GSTM1 null genotype has a statistically significant difference (P = 0.02), while the percentage of GSTT1 null genotype was non-significant (P = 0.76) between the two groups. The percentage of deletion of both genes is higher in professional than non-professional motorcycle drivers. Air pollution in Cotonou by BTEX seems to influence the deletion of the GSTM1 and GSTT1 genes at a higher percentage among professional than non-professional motorcycle drivers.

Keywords

BTEX, Glutathione-S-Transferase, Deletion, PCR, Motorcycle Drivers

1. Introduction

Glutathione-S-transferases (GST) are a family of Phase II enzymes that use reduced glutathione to remove many toxic substances and oxidative stress products [1]. This reduced glutathione (GSH) is the main antioxidant molecule in the cell [2]. GST catalyzes the conjugation of toxic molecules with reduced glutathione and this complex can be expelled from the cell via ATP-binding cassette efflux pumps. In addition, GST is able to detoxify harmful products of cellular metabolism, such as reactive oxygen and nitrogen species through their glutathione peroxidase activity [3] [4]. These enzymes are also involved in other cellular processes such as the regulation of kinase-mediated signal transduction [5] [6]. There are two families of GST in humans: GST present in the endoplasmic reticulum and mitochondria on the one hand and cytosolic GST on the other hand [7]. The cytosolic GST is divided into seven classes: Alpha, Mu, Omega, Pi, Sigma, Theta and Zeta [8]. Allelic variations such as GSTA, GSTM, GSTT and GSTP have been widely described in several studies [9] [10]. Our study is interested in polymorphisms of the Mu and Theta classes, because chronic exposure to BTEX (Benzene, Toluene, Ethylbenzene, Xylene) could compromise the antioxidant capacity of these enzymes and cause oxidative damage such as mutations. The enzymes of GSTM1 and GSTT1 completely lose their activities, when the deletion of their genes is homozygous [11]. Thus, the deletion of GSTM1 and GSTT1, alone or both combined, increases the susceptibility to develop cancer and pathology in various organs such as the liver, lungs, stomach, esophagus, breast, prostate, bladder and the colon [6] [12]. However, very few studies have dealt with the influence of BTEX on GST polymorphisms. Also, the objective of this study is to assess the effect of air pollution containing mostly BTEX on the genes of the GST detoxification enzymes (GSTM1 and GSTT1) in professional and non-professional motorcycle drivers in Cotonou, Benin. The significance of comparing the two groups lies in the amount of time spent in urban traffic by professional motorcycle drivers and depending on this time, BTEX may have a greater effect on the health of professional drivers than non-professional motorcycle drivers.

2. Material and Methods

We recruited sixty (60) motorcycle drivers, including thirty (30) professionals and thirty (30) non-professionals, who were matched according to sociodemographic characteristics following an investigation. Professional motorcycle drivers are those who ride as a profession and are in urban traffic all day while non-professionals are those who are occasionally in urban traffic or just commuting to/from work. This study population was continuously exposed to air pollution containing BTEX and the results were reported in the work of Sagbo *et al.* [13]. Another study showed that the ambient air in Cotonou contains high levels of benzene, carcinogenic compound which come as well as traffic exhaust that gasoline [14].

2.1. Genotyping of the GSTM1 and GSTT1 Genes by Multiplex PCR

DNA extraction by the phenol/chloroform method was carried out using blood preleved in sterile 5 mL EDTA tubes [15]. The quality of the DNA was determined by spectrophometer (Thermo Scientific, Germany) and stored at −20˚C until use.

The multiplex PCR technique was performed to amplify three genes GSTM1, GSTT1 and albumin [16]. Albumin primers were included as an internal positive reaction control. The primers involved in this technique are: GSTM1 (219 bp) F: 5'-GAA-CTC-CCT-GAAA-AAG-CTA-AAG-C-3'; R: 5'-GTT-GGG-CTC-AAA-TAT-ACG-GTG-G-3'; Albumin (350 bp) F: 5'-GCC-CTC-TGC-TAAA-CAAA-GTC-CTA-3' and R: GCC-CTA-AAA-AGA-AAA-AAA-TCG-CCA-ATC and finally GSTT1 (480 bp) F: 5'-TTC-CTT-CTT-ACT-GGT-CCT-CAC-ATC-TC-3' and R: 5'-TCA-CCG-GAT-CAT-GGC-CAG-CA-3' (Eurogentec, France). One hundred (100) ng of DNA were amplified in a 25 µL reaction medium containing 10X PCR buffer, 5 nM of dNTP, $MgCl_2$ (25 mM), 100 µM of each primer (Eurogentec, France) and 5 U of Taq DNA polymerase. PCR was performed using the thermocycler (Mastercycle staff) under the conditions of a cycle of 94˚C for 5 minutes followed by 35 cycles of 94˚C for 1 minute, 64˚C for 1 minute, 72˚C for 70 seconds and a final extension at 72˚C for 5 minutes [16]. The PCR product was subjected to an agarose gel electrophoresis (1.5%) and migrated at 100 V for 55 min, then visualized using a transilluminator. This study has received the consent of the participants and the approval of the Ethics Committee.

2.2. Statistical Method

The data were processed using Statistical Package for Social Science version 20.0 (SPSS20.0). P values less than 0.05 were considered significant.

3. Results

Table 1 in below presents the sociodemographic characteristics of professional and non-professional motorcycle drivers.

The mean age for professional and non-professional drivers was respectively 51.63 ± 5.85 and 49.00 ± 6.92. As for their level of education, 33.33% of both groups had primary; while 26.67% of professional drivers and 30.30% of non-professional drivers had secondary school education. The others ones of the study population, 40% of the professional and 36.67% of the non-professional, are not educated. No significant difference was observed in the age and level of education of the two groups. In addition, all participants are men. These motorcyclists, especially professionals, complain of the faintness, they feel such as fatigue, headache, arthritis, sexual weakness and visual problems related to the inhalation of exhaust fumes which are full of toxic pollutants among which we have BTEX.

The study by Sagbo *et al.* revealed that the means concentrations of benzene, toluene, ethylbenzene and xylene were 77.00 ± 5.45 µg/m^3; 245.91 ± 18.99 µg/m^3;

72.53 ± 7.54 µg/m³ and 67.25 ± 14.54 µg/m³ for professional motorcycle drivers and 25.87 ± 6.51 µg/m³; 139.75 ± 19.08 µg/m³; 64.26 ± 4.99 µg/m³ and 41.60 ± 00.00 µg/m³ respectively for non-professional drivers [13]. These concentrations are very high and higher than the standard accepted by the World Health Organization. We then analyzed the effects of these BTEX on the deletion of the GTM1 and GSTT1 genes. **Figure 1** in below shows the results of electrophoresis after PCR of the DNA of the populations studied.

In this photo above of the electrophoresis gel obtained after PCR, the albumin used as positive control is located at 350 bp, while GSTM1 and GSTT1 are located respectively at 219 bp and 468 bp. The absence of either or both genes in the presence of the positive control indicates a deletion of the GSTM1 or/and GSTT1 genes.

Professional drivers 1Z, 13Z and non-professionals 3T, 13T underwent homozygous deletion of the GSTM1 and GSTT1 genes. Subjects 2Z, 2T have GSTM1 and 15Z has GSTT1 homozygous. Only subjects 27Z and 26T have both genes. Thus, air pollution by BTEX would induce a mutation in the subjects of the study. This mutation was a homozygous deletion that resulted in the loss of these genes in some individuals. **Table 2** shows the genotyping results for the GSTM1 and GSTT1 polymorphism in the professionals and control motorcycle drivers.

Table 1. Sociodemographic characteristics of professional and non-professional motorcycle drivers.

	Professional motorcycle drivers	Non-professional motorcycle drivers	X^2	P-value
Mean age	51.63 ± 5.85	49.00 ± 6.92	15.49	0.560
Primary	33.33%	33.33%		
Secondary	26.67%	30.30%	0.024	0.988
Not in school	40%	36.67%		

Table 2. The percentage of GSTM1 and GSTT1 gene polymorphism in professional and non-professional motorcycle drivers.

GST	Allelic variants	Professional motorcycle drivers N (%)	Non-professional motorcycle drivers N (%)	95 % IC	P-value
GSTM1	Present	09 (30)	18 (60)	−0.59 - 0.00	0.04
	Absent (Null)	21 (70)	12 (40)	0.10 - 1.23	0.02
GSTT1	Present	06 (20)	07 (23)	−0.26 - 0.19	0.76
	Absent (Null)	24 (80)	23 (77)	−0.39 - 0.52	0.76
GSTM1 Null/GSTT1 Null		17 (57)	10 (33)	−0.71 - 0.08	0.01

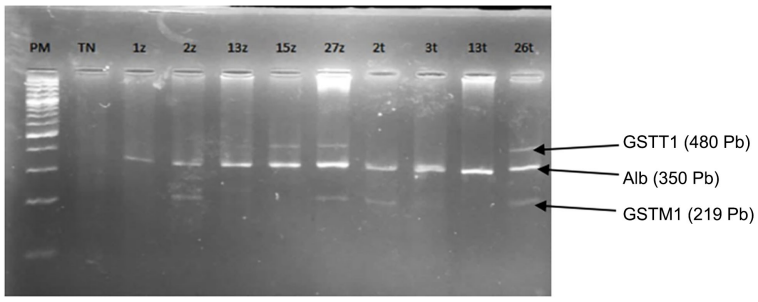

Figure 1. Profile of GSTM1 and GSTT1 genes by multiplex PCR. PM: Molecular weight marker, TN: Negative control, Z: Professional motorcycle drivers, T: Non-professional motorcycle drivers.

The analysis of the **Table 2** shows that the percentage of GSTM1 nulls higher among professional (70%) than non-professional drivers (40%). This increase shows a statistically significant difference (P = 0.02) between the two groups. As for GSTT1, its absence is also more higher among professional motorcycle drivers (80%) than non-professional motorcycle drivers (77%). However, this difference is not statistically significant (P = 0.76). Analysis of the frequency of the absence of the combination of GSTM1 and GSTT1 showed that double deletion is more common among professional drivers than among controls (57% versus 33%).

4. Discussion

In the present study, we evaluated the effects of BTEX on the genetic polymorphisms of GSTM1 and GSTT1 in professional and non-professional motorcycle drivers. A descriptive analysis of the sociodemographic characteristics of these participants indicates that there is no significant difference in the age of professional and non-professional motorcycle drivers (P > 0.05). The sociodemographic results of our study population are consistent with those of Kasemy et al. in Egypt, who worked with motorcycle taxi drivers exposed to benzene compared to unexposed as controls [17]. These two study groups had mean age of 42.80 ± 9.69 and 43.86 ± 6.74 respectively [17] [18] [19]. In contrast, the mean age of professional and non-professional motorcycle drivers in our study in Benin was higher than that of drivers in Iran, Kenya, Nigeria and Brazil, the mean age varied between 24.4 and 33.4 years [20] [21]. This study population is younger than ours. This may be justified by the fact that the motorcycle driving activity practically started in Benin more than 30 years ago. Our results also showed that our study population is made up entirely of men with a low level of education. These results are consistent with those of Ayi-Fanou *et al.*; Fourn and Fayomi [22] [23]. Similarly studies have shown that motorcycle taxi driving activity is practiced almost exclusively by men with little education [24] [25] [26]. Our study population is exposed to BTEX differently depending on whether they are professional or non-professional motorcycle drivers.

According to the work of Sagbo *et al.* exposure to BTEX was higher among

professional drivers than non-professional drivers [13]. The benzene level among professional drivers is fifteen times higher than the standard accepted by the World Health Organization while it is five times higher than this standard among non-professional drivers. Previous work has shown that BTEX influences levels of C-reactive protein and blood cells. Regarding the influence of BTEX on the genes responsible for the detoxification enzymes (GSTM1 and GSTT1), our results showed that the percentage of absence of GSTM1 was higher among professional drivers than non-professional with a statistically significant difference ($P = 0.02$). Professional and non-professional drivers are all at risk, but professionals are more at risk. Both groups are able to develop several pathologies [6] [12]. Knudsen *et al.* reported significant prevalence of GSTM1 null among bus drivers exposed to air pollution [27]. A cohort study of schoolchildren in Durban, South Africa, showed a GSTM1 null rate (27%) lower than that of our study [28]. Nielsen *et al.* showed that deletion of GSTM1 could lead to higher adduct levels in bus drivers exposed to air pollution [29]. Similarly, our results on GSTT1 reveal that there is more deletion of GSTT1 null among professional drivers than non-professionals. However, this difference is not statistically significant ($P = 0.76$). In addition, the results of this study showed that the percentage of the combination of the absence of the two genes GSTM1/GSTT1 is higher among professional motorcycle drivers (63%) than non-professional (33%). Thus, the combination of the absence of the two genes in an individual is a major risk factor for the development of certain pathologies [6] [12]. Studies carried out in Benin by Avogbe *et al.* showed that the absence of GSTT1 was associated with poor urinary excretion of the non-toxic metabolite benzene S-phenylmercapturic acid, which may suggest that GSTT1 modulate the detoxification of benzene [30]. The work of Sirivarasai *et al.* showed that the presence of pollutants in the blood induces the deletion of GSTM1 and GSTT1 and leads to inflammatory responses [18].

5. Conclusion

The entire study population is exposed to very high concentrations of BTEX above the standard accepted by the World Health Organization. The entire population of Cotonou is at risk of developing diseases due to the deletion of the GSTM1 and GSTT1 genes responsible for detoxification toxic compounds. But professional drivers are more at risk than non-professional because of the large deletion observed in their group. It is therefore urgent to take certain measures to improve the air quality in Cotonou.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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