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POLYMORPHISMS OF GSTM1, GSTT1 AND GSTP1 AND THEIR POSSIBLE ASSOCIATION WITH THE DEVELOPMENT OF DENTAL CARIES. PILOT STUDY

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ABSTRACT

Objective: The purpose of this investigation was to study the relationship between the polymorphisms of GSTM1, GSTT1 and GSTP1 with the susceptibility to dental caries in Mexican. **Methods:** In a group of 64 individuals, the DMFT index and GST polymorphisms were determined and related. **Results:** The frequencies for GSTM1 were 48.4% with the wild allele and 51.6% null, with a mean DMFT of 6.1 and 5.5 respectively. For GSTT1 were 73.4% with the wild allele and 26.6% null, with a mean DMFT of 6.6 and 5.8, respectively. In GSTP1 exon 5, 23.4% with the wild allele, 53.2% (a/b) and 23.4% (b/b), the mean DMFT was 4.3, 6.6 and 5.9 respectively. 100% had the GSTP1 exon 6 wild genotype, with a mean DMFT of 6.4%. In the combined genotypes, the lower DMFT corresponds to the GSTM1 wild type genotype, GSTT1 wild type, GSTP1 exon 5 wild type, GSTP1c wild type and the highest DMFT value to GSTM1 null genotype; GSTT1 null; GSTP1 exon 5 a/b; GSTP1 exon 5 a/a. **Conclusions:** The results show the possible association between GST polymorphisms and the susceptibility to develop dental caries due to alteration of the enzymatic activity, this provides evidence that the genetic load may be a risk factor to dental caries.

KEYWORDS: Caries, Glutathione S-Transferase, Polymorphisms, Oxidative Stress & DMFT

1. INTRODUCTION

The WHO estimated in 2012 that 60–90% of children in the world have caries and almost 100% of adults do (WHO, 2012). In Mexico, the Epidemiological Surveillance System of Oral Pathologies reported in 2015 that in several states of the Mexican Republic, 93.2% of the population had caries (SIVEPAB, 2015).

Dental caries is a chronic and multifactorial infectious disease, as a result of damage to the pulp, tooth enamel and mineralized dentin. The bacterial infection of the enamel and dentin induces an inflammatory response in the dental pulp (pulpitis), due to the recognition of bacterial components by odontoblasts and pulp cells, triggering an antibacterial, immune and inflammatory response, which cause the production of cytokines and reactive oxygen species (ROS; Chang et al., 2005; Aksoy, 2011; Monisha & Savitha, 2016). If the infection is mild, pulp damage can be cured with dental treatment. However, it is common for the inflammation state to remain after treatment and enter a chronic state. When this occurs, the increase in the level of superoxide (O_2) and hydroxyl groups (OH) causes damage to bacterial cells, hosts and surrounding tissues. To avoid excessive damage caused by the production of ROS, dental cells and neutrophils have antioxidant mechanisms that eliminate them and decrease cell and tissue damage. Among these, it is that of the isoenzymes

encoded by the family of genes Glutathione S-transferase (GST; Matsui et al., 2009; Aksoy, 2011; Farges et al., 2015; Monisha & Savitha, 2016).

The family Glutathione S-Transferase (GST), responsible for the metabolism of xenobiotics in phase II, protects cells from oxidative damage by the conjugation of glutathione with electrophilic substrates (Hayes et al., 2005; Wu & Dong, 2012). Currently, the most studied genes of the GST family are Mu (μ), Pi (π) and Theta (θ). GSTM1 (μ), which is located on chromosome 1 region p13.3, has eight exons with a length of 4.2 kb and four allelic variants: A (wild), B, C and 0 or null. GSTT1 (θ) is located on chromosome 22p region 11.2, has six exons and two allelic variants: wild GSTT1A and GSTT1-0 or null. GSTM1 (0) and GSTT1 (0) results in the loss of enzymatic activity (Hayes et al., 1995; Rodríguez et al., 2013). GSTP1 (π) gene is located on chromosome 11 region q13, has nine exons, with a length of 3.2 kb and has three allelic variants: GSTP1a, GSTP1b, and GSTP1c. The wild allele is GSTP1a. The allele GSTP1b is polymorphic, is found in exon 5; it is the result of the substitution of a base, from ATC (Ile) to GTC (Val) in codon 104. The third polymorphism, GSTP1c, is found in exon 6 at codon 113, a result of a substitution of a base from GCG (Ala) to GTG (Val) (Osman et al., 2011; Mejia et al., 2013).

The presence of polymorphisms in genes encoding for the GST isoenzymes can alter their enzymatic activity and influence the ability to eliminate ROS produced by the inflammatory response. This may be associated with damage to dental pulp and susceptibility to develop tooth decay (Bonola et al., 2015; Farges et al., 2015; SIVEPAB, 2015). However, the study of polymorphic genes involved in the development of caries has been little explored. The purpose of this investigation was to know for the relationship between the polymorphisms of GSTM1, GSTT1, and GSTP1 with the susceptibilityto dental caries in individuals of Mexican origin.

2. MATERIAL AND METHODS

2.1 Study Group

This is a cross-sectional study. An invitation to participate was extended to individuals visiting the Centro de Investigación en Ciencias Médicas, Universidad Autónoma del Estado de México, from January 2017 to January 2018. Participation was voluntary. Only adults with 20 or more permanent or temporary teeth were included. The study group was 64 individuals.

2.2 Ethical Considerations

This study was approved by the Ethics Committee of the Centro de Investigación en Ciencias Médicas from Universidad Autónoma del Estado de México. All procedures performed in studies involving human participants were in accordance with the ethical standards of the 1964 Helsinki Declaration. All participants in this study voluntarily accepted and signed an informed consent letter.

2.3. Dmft Index Evaluation

The determination of caries in the individuals in the study was by means of the DMFT Index. It is the average of permanent decayed, lost and sealed teeth. This was calculated based on 28 permanent teeth, excluding the third molars. The value was obtained from the sum of permanent decayed, lost and sealed teeth, among the total number of pieces examined (Nithila et al., 1998; Aguilar et al., 2009).

2. 4 Dna Extraction

A 4 mL peripheral blood sample was taken in a Vacutainer tube with heparin**.** The DNA extraction was performed from the blood samples, using the commercial kit 'Quick-g DNA Mini Prep kit" (Zymo Research).

2.5 Genotyping of gstt1 and gstm1

For the identification of GSTT1 and GSTM1 polymorphisms it was used endpoint PCR with a final volume of 50 µL and using CYPA1 as control gene. The mixture had 5 µL of 5x PCR buffer (Promega), 4 µL of 25 mM $MgCl_2$ (Promega), 1 µL of dNTPs (Fermentas), 32.7 µL of molecular biology grade H_2O, 0.3 µL Taq polymerase 5 U/µL (Promega), 2 µL of template DNA and 1 µL of each primer: CYP1A1-f, CYP1A1-r, GSTM1-f, GSTM1-r, GSTT1-f and GSTT1-r 30 pM/µL. The thermocycler program were 94°C for 5 minutes, 35 cycles of 94°C for 45 seconds, 62°C for 45 seconds, 72°C for 45 seconds and 72°C for 5 minutes. The determination of polymorphisms was by electrophoresis on agarose. GSTT1 wild type is the 450 pb fragment and the absence of this fragment indicates GSTT1 null. For the GSTM1 wild type is the 215 pb fragment and the absence of this fragment is GSTT1 null. A fragment of 312 bp is CYP1A1 (Montero et al., 2007; Rodríguez et al., 2013).

2.6 Genotyping of gstp1

The identification of GSTP1 polymorphisms exon 5 and exon 6 was performed in two different PCRs with a final volume of 25 µL. The mixture had 2 µL of DNA, 5 µL of 5x PCR buffer (Promega), 4 µL of 25 mM $MgCl_2$ (Promega), 0.3 µL of 5 U/µL Taq Polymerase (Promega), 0.5 µL of 25 µM dNTPs (Fermentas), 13.7 µL of molecular biology grade H_2O and 1 µL of each primer GSTP1b-f, GSTP1b-r or GSTP1c-f GSTP1c-r (30 pM/µL). For GSTP1c, the thermocycler program were the same as for GSTT1 and GSTM1. The thermocycler program for GSTP1b were 94°C for 5 minutes, 94°C for 45 seconds, 62°C for 45 seconds, 72°C for 45 seconds and 72°C for 5 minutes for a total of 35 cycles (Mejia et al., 2013).

Enzymatic digestions were using the BsmAI enzyme (Fermentas) in exon 5 and the AciI enzyme (Fermentas) in exon 6 with a final volume of 10 µL. The mixture consisted in 7 µL of molecular biology grade H_2O, 2 µL Digest Green buffer (Fermentas), 1 µL Enzyme and 10 µL of PCR product. The digestion incubated temperature were 37°C for 8 hours. The determination of polymorphisms was by electrophoresis on 2% agarose. For exon 5, the presence of the fragments of 176 bp, 91 bp, and 85 bp correspond to heterozygous GSTP1b. Two fragments, one of 91 bp and another of 85 bp correspond to homozygous GSTP1b. A single 176 bp fragment is wild GSTP1. For the exon 6 polymorphisms, the 332 bp fragment is homozygous GSTP1c. Three fragments, 332 bp, 174 bp, and 158 bp are heterozygous GSTP1c and two fragments, 158 bp and 174 bp are wild GSTP1 (Mejia et al., 2019).

2.7 Statistical Analysis

Statistical analysis was performed in search of intra-gene differences that are between the polymorphisms of each gene. Mann–Whitney U test was applied for GSTM1 and GSTT1 and Kruskal–Wallis for GSTP1, using the Sigma Stat 3.5 program.

3. RESULTS

3.1 Sociodemographic Characteristics

A total of 64 individuals, 24 men, and 40 women were participated in the study. The average age was 35.5 ± 15.3 years and all were from the State of Mexico.

3.2 Polymorphisms of the gst and dmft Index

The frequency of the genotypes found for the GSTM1 gene was 48.4% (31 individuals) for the wild allele, and 51.6% (33 individuals) for the null allele, with a mean DMFT of 6.1 and 5.5 respectively. For the GSTT1 genotypes, 73.4% (47 individuals) for the wild genotype y 26.6% (17 individuals) for the null allele, with a mean DMFT of 6.6 and 5.8, respectively. For GSTP1 exon 5, a frequency of 23.4% (15 individuals) for the wild genotype (a/a), 53.2% (34 individuals) heterozygous (a/b) and 23.4% (15 individuals) homozygous (b/b), the mean DMFT was 4.3, 6.6 and 5.9 respectively. For GSTP1 exon 6, 100% (64 individuals) had the wild genotype and the mean DMFT of 6.4. Statistical analysis using the Mann–Whitney U test for GSTM1 and GSTT1, Kruskal–Wallis for GSTP1. None of the comparisons showed a significant difference. Table 1 shows detailed data for each gene, the corresponding DMFT index average ± the standard deviation and the value of *p*.

Table 1: DMFT Index and the Polymorphisms of each of the GST Genes

Gen	Genotype	n (%)	Decayed ẋ±SD	Lost ẋ±SD	Sealed ẋ±SD	DMFT ẋ±SD	*p*
GSTM1	Wild	31(48.4)	2.9±0.8	1.5±1.1	2.9±0.3	6.1±1.2	0.64
	Null	33(51.6)	2.4±1.8	0.7±.1	2.4±0.9	5.5±1.4	
GSTT1	Wild	47(73.4)	2.2±0.4	1.1±0.9	2.7±1.1	6.6±1.2	0.94
	Null	17(26.6)	2.3±1.2	1.1±0.8	2.4±0.7	5.8±1.1	
GSTP1 (exon 5)	a/a	15(23.4)	1.1±0.6	2.1±2.9	3.1±3.7	4.3±0.8	0.32
	a/b	34(53.2)	3.3±0.3	0.7±0.6	2.6±1.6	6.6±1.2	
	b/b	15(23.4)	2.7±1.2	0.9±0.3	2.3±0.9	5.7±1.3	
GSTP1 (exon 6)	a/a	64(100)	2.6±0.3	1.1±0.9	2.6±1.1	6.4±1.5	NS

Statistical analysis using Mann–Whitney U test for GSTM1 and GSTT1, Kruskal–Wallis for GSTP1. NS: Not significant.

3.3 Combined gst Genotypes and dmft Index

We found 12 different genotypes combined in the participants. The most frequent genotype was GSTM1 null; GSTT1 wild type; GSTP1 exon 5 a/b; GSTP1 exon 6 wild type with 28%. The least frequent was GSTM1 null; GSTT1 null; GSTP1 exon 5 b/b; GSTP1 exon 6 wild type and GSTM1 null; GSTT1 null; GSTP1 exon 5 a/b; GSTP1 exon 6 wild type with 1.5% for each one. The frequencies of combined genotypes are shown in Table 2.

Table 2: Frequencies of Combined GSTM1, GSTT1, and GSTP1 Genotypes

Number	Genotypes				n (%)
	GSTM1	GSTT1	GSTP1 exon 5	GSTP1 exon 6	
1	null	wt	a/b	wt	17 (28)
2	wt	wt	a/b	wt	11 (18)
3	null	wt	b/b	wt	6 (9)
4	null	wt	wt	wt	6 (9)
5	wt	null	a/b	wt	5 (7)
6	wt	wt	b/b	wt	4 (6)
7	wt	null	wt	wt	4 (6)
8	wt	null	b/b	wt	4 (6)
9	wt	wt	wt	wt	3 (5)
10	null	null	wt	wt	2 (3)
11	null	null	b/b	wt	1 (1.5)
12	null	null	a/b	wt	1 (1.5)

wt: wild type

The average DMFT index for each of the 12 different genotypes was determined. They were organized according to the magnitude of the DMFT index. Table 3 shows the results, ordered from the lowest to the highest DMFT index. It was observed that the lower DMFT value corresponds to the combined genotype that presents the wild alleles in the genes (GSTM1 wild type, GSTT1 wild type, GSTP1 exon 5 wild type, GSTP1c wild type), with a DMFT value of 3. The highest DMFT value (12) corresponds to a genotype GSTM1 null; GSTT1 null; GSTP1 exon 5 a/b; GSTP1 exon 5 a/a.

Table 3: Combined GSTM1, GSTT1 and GSTP1 Genotypes and DMFT Mean Value

Number	GSTM1	GSTT1	GSTP1 Exon 5	GSTP1 Exon 6	DMFT Mean
9	wt	wt	wt	wt	3
3	null	wt	b/b	wt	3.5
7	wt	null	wt	wt	4
4	null	wt	wt	wt	4.5
5	wt	null	a/b	wt	5
2	wt	wt	a/b	wt	6
1	null	wt	a/b	wt	6
8	wt	null	b/b	wt	6
10	null	null	wt	wt	6.5
6	wt	wt	b/b	wt	7
11	null	null	b/b	wt	9
12	null	null	a/b	wt	12

wt: wild type

Since the number of individuals for each of the combined genotypes is very small, the results of the statistical analysis cannot be considered as reliable. However, a tendency to susceptibility to develop caries was observed in the presence of the polymorphic versions of the three genes.

4.1 DISCUSSIONS

Caries is an irreversible and cumulative disease of multifactorial etiology (Kojima et al., 2013). Recent studies have proposed that the gene load is a possible factor, associated with the development of dental diseases (Wang et al., 2012;

Shimizu et al., 2013). In addition to this, the expression of antioxidant enzymes and cytokines are associated with a state of inflammation of the oral tissue and may participate in the progression of dental diseases (Aksoy, 2011).

There are few studies on the association of GST gene family and enzymes with dental diseases. One of the works in this regard was carried out by Camargo et al. (2014), who studied the influence of polymorphisms of the GSTM1, GSTT1 and GSTP1 genes on the susceptibility to develop periodontitis by smoking and non-smoking individuals. They found an association between the development of this disease and the null GSTM1 genotype. On the other hand, Varghese et al. (2012), evaluated the levels of GST enzymatic activity in gingival crevicular fluid (GCF) and gingival tissues of patients with chronic periodontitis, finding a significant increase in the enzymatic activity of patients when compared with a control group. The results obtained in these works show the association between polymorphisms and enzymatic activity of GST and the development of periodontitis. This is relevant since the polymorphisms of GSTM1, GSTT1 and GSTP1 can alter the enzymatic activity of total GST in cells and tissues subjected to oxidative stress or other agents (Mejia et al., 2017; Mejia et al., 2018) and this translates as susceptibility to periodontitis. However, the results obtained in the present investigation differ from the works in periodontitis, since individually the polymorphisms of each gene were not associated with the development of caries.

On the other hand, it is pertinent to consider that the enzymatic activity of GST in the cell is carried out jointly and not individually (Rodríguez et al., 2013; Mejia et al., 2017). This is relevant since in this work, when analyzing the combined genotypes of the three genes, we find differences between genotypes 6 and 9. In addition to the above, it is observed that genotypes 11 and 12 that have polymorphisms in all three genes have the highest DMFT index. The analysis of the combined genotypes suggests that the presence of polymorphisms in genes involved in the elimination of ROS, such as those of GST, can influence the development of dental diseases and high DMFT value.

Based on the results obtained in this research, and in order to demonstrate the role that polymorphisms of the GST genes play in the susceptibility to dental diseases, the study should be continued in a larger group, which would allow for obtaining significant results. The present research supports the participation of the gene load as a factor in the development of dental caries, which is determined by the interaction of various factors.

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6. CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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