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Association of HlA Allele and Level of Streptococcus Mutans in Saliva of Normal and Mentally Retarded Egyptian Children

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Abstract: Streptococcus Mutans(SM) is thought to play a major role in the etiology of dental caries. The aim of this study was to evaluate the relationship between HLA DRBI*0701-DRBI*0702 and DRBI*1101-DRBI*1104 loci and levels of SM in normal and mentally retarded (MR) Egyptian children. Subjects were 68 normal and 58 MR children, their age ranged from 6 to 14 years. Results showed significant association between caries level and HLA DRBI*0701-DRBI*0702 in MR children, as well as no significant association between DRBI*1101-DRBI*1104 allele and caries level in both groups. In conclusion, the results support the hypothesis of the association between HLA classII genetic profile and colonization of SM as pathogens for dental caries. However, further studies are needed to evaluate other HLA alleles as genetic variants in dental caries.

Key words: dental caries, HLA, streptococcus mutans.

INTRODUCTION

Dental caries is a wide spread infectious disease, It is a major public health problem particularly among younger children. It is important for preventive strategies to predict factors influencing the development of dental caries at the earliest possible stage in their life (Nariyama, *et al*., 2004). It affect the majority of people but about 3% of them seem to be resistant (Lehner, *et al*., 1981).

Streptococcus Mutans has been implicated as a principle etiologic agent for dental caries (Smith, 2002). The overall prevelance of SM colonization in infants without teeth was 64% (Wan, *et al*., 2001) 84% of infants harbored the SM bacteria by 24 ms of age (Wan, *et al*., 2003). More than 85% of the adult population is colonized although there are a wide individual differences in the level of colonization (Togelius and Bratthal, 1982; Salonen, *et al*.,1990). It was observed that 25% of genetically identical monozygotic twins were subjected to colonization by SM prior to emergence of the first tooth (Bockmann, *et al*., 2011). These cannot be fully explained by environmental factors such as retention sites, diet and oral hygiene. Although properties of saliva (Tenovuo, 1987) diet (Kristofferson and Birkhed, 1987) and oral hygiene (Axelsson, *et al*., 1987) are some factors well known to affect the colonization of mutans streptococci do not fully explain the inter-individual differences in colonization levels (Wallengren, *et al*., 2001).

Twins studies have shown that genetic factors contribute to caries susceptibility (Shuller, 2001).

The first genome-wide scan for caries was performed in forty six families in the Philippines, similarly influenced by confounding factors such as diet, oral hygiene habits, fluoride exposure, access to dental care and with similar cultural and behavioral habits. Suggestive loci for low caries susceptibility (5q13.3, 14q11.2, and xq27.1) while for high caries susceptibility (13q31.1 and 14q24.3). Genes that may be related to salivary flow and diet preferences are proposed as possible candidates (Vieira, *et al*., 2008).

Moreover if one accepts that caries susceptibility is in part influenced by the oral micro biota. The one's immune response capability may also modulate this process (Napimoga, *et al*., 2004). Helper T lymphocytes recognize fragments of foreign (or self) antigens in the peptide binding cleft of HLA class II molecules which are cell membrane glycoprotein, their activation is a crucial step in the induction of many immune and auto immune responses (Ong, *et al*., 1991). It was demonstrated that HLA molecules were generally associated with the early phase of the immune response to caries infection (Yoshiba, *et al*., 1996).

The HLA surface molecules are specified by the HLA genes on the short arm of six chromosome (Krangel, *et al*., 1980) and has high polymorphism (Ong, *et al*., 1991). The binding of a specific peptide by HLA classII molecules is more likely to be linked to DRBI alleles, as they define the amino acid sequence of the antigen binding sites on the classII molecules (Hughes, *et al*., 1996).

The association of HLA alleles with the disease is controversial, many studies suggest that the immune responses of SM showed significant association with HLA classII genes (Lehner, *et al*., 1981; Gonwa, *et al*., 1983; Yoshiba, *et al*., 1996; Acton, *et al*., 1999; Wallengren, *et al*., 1991,2001). While others found no significant associations (Wallengren, *et al*., 1997; Altun, *et al*., 2008).

A high dose of purified streptococcal antigen was required to stimulate release of T helper activity from cells that were HLA-DR4 compared to other HLA phenotype. In contrast a low dose of antigen was required to

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stimulate release of T helper activity from cells carrying the HLA DR1,2,3,6 cross reactive groups (Lehner, *et al*., 1981).

Acton, *et al*., (1999) found that the frequency of DRBI*9 and 11 were decreased in African American woman with high compared to low level of SM colony forming units.

Moreover another study was performed by Chiba, *et al*., (2005) to examine whether the salivary levels of SM differ between DMFT≥10and DMFT=o subjects according to HLA DRBI alleles, the results suggest that saliva levels of SM differ between DMFT≥10 and DMFT=o subjects by specific HLA-DRBI alleles.

Several explanations exist for the discrepancies found between various studies, since the products of the HLA system were serologically defined in previous studies whereas in recent studies the HLA were determined by PCR. Also various HLA alleles differ in their frequencies among different ethnic groups Vlachoyiannopoulos, *et al*., (2000). Wallengren, *et al*., (2001) stated that the variable level of correlation demonstrated for SM colonization should be thought on a subgroup level. They demonstrated that degree of immunologic reactivity was more pronounced among subgroup levels.

The present study aimed to evaluate the relationship between HLA-DRBI*0701-*0702 alleles DRBI*1101-1104 alleles and levels of SM in saliva of normal and mentally retarded Egyptian children.

Subjects and Methods:

The study included 126 Egyptian children (68 normal children recruited from the out patients of pedodontic department, Faculty of Oral and Dental Medicine, Cairo University. 58 mentally retarded children from the out patients clinic of the Human Genetics Department NRC). Their age ranged from 6 to 14 years.

Subjects had no history of antibiotic use for the last six months and they were subjected to the following:

1-Dental caries examination was performed following the criteria listed by the WHO, (1985). The DMFT was performed for the permanent dentition and the dft for the primary dentition.

Subjects were grouped according to dental caries examination into: a) Those who are caries free (no carious teeth or filled or missed due to caries or indicated for extraction due to caries). b) Children with high caries scores (>6 carious or filled or missed due to caries or indicated for extraction due to caries).

Sample Collection:

a) For PCR study:

Saliva or buccal smear samples were taken on saline solution to obtain DNA from buccal epithelial cells. Subject rinsed their mouths with 15 ml (0.9%) saline solution, taken in three rinses for about 30 second and collected in 50ml falcon tubes. For subjects who are unable to rinse samples were collected by sweeping the inner surface of the cheek by a spatula and rinsing out the cells in 0.9% saline solution.

b) For microbiological study:

Saliva samples were collected as follows: a piece of paraffin wax, of approximately 1-5g was supplied to the child. Each individual chewed the paraffin wax for about 30 secs. Approximately 2.0 ml of stimulated saliva were collected in sterile glass tubes. Saliva samples were transported to the microbiology laboratory on ice for processing within 2 hours of collection.

Methods:

1. Polymerase chain reaction for the study of HLA DRBI*0707-0702 and DRBI*1101-1102 alleles:

a- DNA Extraction:

Buccal epithelial cell were pelleted by centrifugation at 500g for 10mins. The cell pellet was resuspended in 200-400ul (50 mM NAOH), according to the size of the pellet. Samples were heated at 100 ˚C for 10min. The suspension was neutralized in 30-60ul Tris HCL (PH8). Cellular debris was then pelleted by centrifugation in a microfuge for 1min.The supernatant was transferred into autoclaved ependorf tube. Five microleters of the supernatant were used for polymerase chain reaction amplification with sequence -specific primers (PCR-SSP).

b) PCR-SSP:

Design of primers using those published according to (Olerup and Zetterquist, 1992). Primer mix 7 and 11 to identify HLA alleles, DRBI*0701-0702 and DRBI*1101-1104 respectively.

Primer mix7 amplifies 232bp product assigning DRBI*0701-0702. Primer mix11 amplifies a 176bp product assigning DRBI*1101-1104. A primer pair C5 and C3 included as an internal positive amplification control it amplifies 796bp product.

The PCR reaction mixtures considered 100ng genomic DNA in 2ul PCR buffer, 200uM of each dATP dCTP, dGTP, and dTTP, 1uM of the allele and group DRB primers, 0.2uM of the control primer (C3 and C5) and 0.5unit of amplitac diluted 1 to 10 in 1xPCR buffer. Each reaction was performed for 33 temperature cycles

(94 ˚C-20 sec, 65 ˚C-50sec, 72 ˚C-20 sec). The PCR generated SSP products were subjected to electrophoresis in 1.5% agarose gel and stained with ethedium bromide. Gels were examined under UV illumination.

2) For Microbiological Study:

Selective media (mitis-salivarius agar has been used for organism isolation). Samples were diluted in tenfold steps. Homogenization done by vortex mixer. From each dilution, 0.1 ml were spread on freshly prepared media. Colony forming units (CFU) were calculated.

- For detecting and identifying streptococcus mutans, direct microscopy was used. Morphological type studies, cultivation and enzyme tests were performed.

3) Statistical Method:

Differences between groups were assessed statistically with Mann-Whitney test. P<0.05 was considered significant.

RESULTS AND DISCUSSION

A total of 126 Egyptian children (68normal children: 36 males & 32 females and 58 mentally retarded children: 25 males & 33 females) were included in the study. Their age ranged from 6 to 14 years.

DNA was extracted from saliva. The association of DRBI studied alleles and level of SM in normal and mentally retarded children are shown in table 1. It was observed that absence of DRBI*0701-0702 alleles for normal children was significantly associated with high level of SM compared to low level for normal children who possessed the allele (p<0.05) while high SM was positively associated with absence of DRB* 1101-1104 in saliva of MR children R(P<0.05).

Table 1: The association of DRBI studied alleles and mean, SD of SM levels in saliva of normal and mentally retarded Egyptian children.

parameter	Allele absence Mean ± SD	Allele presence Mean ± SD	p
Salivary level of SM and DRBI*0701-0702 allele for normal children	107±129.15	12.57±22.76	0.002*
Salivary level of SM and DRBI*0701-0702 allele for MR children	39.6 ±97.71	15.63 ±26.3	0.373
Salivary level of SM and DRBI*1101-1104 allele for normal children	133.83±152.1	35.84 ±77.73	0.066
Salivary level of SM and DRBI*1101-1104 allele for MR children	84 ±127.44	9.03 ±10.92	0.013*

* = statistically significant

Frequency, percentage and P value of DRBI*0701-0702 alleles for mentally retarded children with free and high caries level are shown in table 2 and present significant statistical difference (P<0.05).

Table2: Frequency, percentage and P value of DRBI*0701-0702 alleles for mentally retarded children with free high caries level.

	DRBI*0701-0702				P
	absence	%	Frequency	presence	
mentally retarded children free from caries (n=20)	6	33.3	12	66	
mentally retarded children with high caries level (n=20)	20	100	0	0	0.016*

P<0.05= significant difference

No significant association between DRBI*1101-DRBI*1104 allele and caries level in normal and mentally retarded children was observed (P>0.05).

Discussion:

Caries is an etiologically complex disease process. It is likely that numerous microbial, genetic, immunological, behavioral, and environmental contributors are at play in determining the occurrence and severity of clinical disease Gati and Vieira, (2011). The magnitude each of these factors contributes to caries can vary significantly on an individual basis. The relative contributions of genetics and environment in each of these domains have not been defined Shuller, (2001).

One aspect of genetic effects is genetic modification in immune response. Individuals with either inherited or acquired immune deficiency are subject to increased risk for dental caries (Magidan, *et al*., 1996).

HLA classII molecules play an important role in immune responsiveness and different hypothesis can explain their role. When cariogenic microorganisms enter the body, antigen presenting cells first process them. These processed antigens (as peptides) are linked to the HLA classII molecules and this complex is presented to T-helper cells of the immune system, which activate the immune response.

Since mutans streptococci are found in almost all individuals, the large differences in oral colonization levels between individuals can be explained by variations in the immune response some types of MHC molecules stimulate B-lymphocyte cells effectively and produce low salivary IgA activity against mutans

streptococci Wallengren, *et al*., (2005). A likely explanation might be that the alleles have an amino acid sequence that binds poorly to the antigenic peptide of the mutnas streptococcus Bagherian, *et al*., (2008).

The study showed positive association for the abscence of DRBI*0701-0702 allele and high level of SM in saliva of normal children, while high SM was positively associated with abscence of HLA DRBI*1101-DRBI*1104 allele in saliva of MR children.

Chliderstone, *et al.,* (1991) demonstrated that level of T cell in vitro proliferative response to antigenic fragment from SM was related to the DR allele presenting the peptide. The T cells from individuals who were DRBI*4,5 or 6 required a larger size synthetic SM peptide to generate a significant Tcell response than Tcells possessing other DRBI alleles.

High level of SM CFUs in African American woman were positively associated with absence of DRBI*11 allele compared to low level of SM in those who possess the allele although these differences were not statistically significant (Acton, *et al*., 1999).

(While Chiba, *et al.*, 2005) suggest that saliva level of SM differ between subjects who had high DMFt>/10 compared to those with DMFT=0 by specific HLA-DRBI alleles.

The absence of DRBI*0701-0702 allele in MR children was significantly associated with high caries index. We didn't observe an association for any of the assessed alleles with caries index for normal children.

An association of caries scores with HLA-DRBI alleles was not observed in the study on military recruits from Netherland DeVries, *et al*., (1985). In the study carried by Acton, *et al.,* (1999) on African American woman they observe no significant association between DRBI allele and DMFS.

Altun, *et al.,* (2008) showed no significant difference between the frequency of HLA alleles in high caries children (dft and DMFT> or =5) and HLA alleles in caries free children (dft and DMFt=0).

Also Yuki, *et al.,* (2001) compared HLA- DRBI allele -DRAI and DQBI allele frequencies between caries free and caries susceptible in 100 unrelated Japanese healthy young adults. None of the HLA classII frequencies showed significant differences between the caries free and caries susceptible group.

All these studies were in accordance with the result obtained for normal children.

While mentally retarded children results may be attributed to the fact that caries formation depends on many other factors in addition to level of cariogenic organisms and immune responsiveness such as tooth anatomy, diet and overall health.

Lehner, *et al.,* (1981), studied the distribution of HLA DR antigens in a group of 24 individuals with high or low DMFS indices. Their study revealed that HLA DRW 6-1,2,3 had a significant relationship to the DMFS index and to low dose response to SM antigens.

Olerup, (1991) stated that the penetrance of the disease in individuals carrying the associated allele is of the low in many diseases which can be attributed to environmental factors and also to the influence from other genes.

It was reported that the genes within HLA complex were associated with altered enamel development and increased susceptibility to dental caries. The enamel defect may play a specific role in caries development Aine, *et al*., (1990); Mirriani, *et al*., (1994); Aiguire, *et al*., (1997).

The results support the hypothesis of the association between host classII genetic profile and colonization of SM.

There is a clear statement that in the presence of large biological and environmental variability genetic effect can differ across different populations or even among generations within the population so the diagnostic use of genes might be limited to a specific population and may not apply globally or across an ethnic group Yoshie, *et al*., (2007).

Future management of dental caries requires early detection and risk assessment if the profession is to achieve timely and cost-effective prevention and treatment for those who need it most.

Further investigations are required to study the association of other HLA alleles with SM levels and caries experience in Egyptian population.

Summary and Conclusions:

- High levels of SM were positively associated with absence of HLA-DRBI*0701- DRBI*0702 in normal children while in mentally retarded children high level of SM were positively associated with absence of HLA-DRBI*1101-1104 alleles.
- The results support the hypothesis of the association between host HLA class II genetic profile and colonization of SM.
- A significant association was observed between caries level and HLA-DRBI*0701-0702 in MR children.
- However, further studies are needed for confirmation and to evaluate other HLA alleles as genetic variants in dental caries.

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