

Relationship Between Salivary Composition and Dental Caries among a Group of Egyptian down Syndrome Children

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Abstract: Dental caries is a complex disease, the etiology of which has received significant research attention during the nineteenth and most of the twentieth centuries. Some syndromes which have chromosomal abnormalities reported to be associated with low caries indices. Down syndrome is an example of this condition; however, the reason of the low incidence of caries in the Down syndrome is unclear. Saliva can affect incidence of dental caries in four general ways, firstly as a mechanical cleansing which result in less accumulation of plaque, secondly by reducing enamel solubility by means of calcium, phosphate, and fluoride, thirdly by buffering and neutralizing the acids produced by cariogenic organisms or introduced directly through diet and finally by anti-bacterial activity. The aim of this study is to investigate the relationship between various salivary composition and dental caries in Down syndrome children. A total of 30 Egyptian children were included in this study, their ages ranged from eight to fourteen years, they were divided into two groups (Down syndrome, n= 20 and normal control group, n = 10). Dental caries was assessed according to the method described for basic oral health surveys by the world health organization. For each child 5 ml saliva was collected and divided as follows, 1 ml for testing PCR and IgA, 1 ml for testing electrolytes, 2 ml for testing pH and viscosity and 1 ml for culturing of streptococcus mutans bacteria. The result of this study revealed that caries incidence in children with Down syndrome was lower than that of the control group. Down syndrome children have statistically significant higher mean values of salivary immunoglobulin A than that of normal children ($P < 0.001$). Salivary pH was significantly elevated in Down syndrome children (6.84) than in the control group (6.5). Viscosity of saliva and streptococcus mutans count in Down syndrome children was lower than in control group. All the salivary electrolytes are significantly elevated in Down syndrome children. Based on this study we can conclude that the low caries incidence in children with Down syndrome can be related to its salivary alkaline pH, lower viscosity, lower St. mutans count and significant rise in its salivary immunoglobulin A than that of the normal children.

Key words: Dental caries, Down syndrome, Saliva

INTRODUCTION

Dental caries development is considered to involve a triad of indispensable factors which can be concluded as bacteria in dental plaque, carbohydrates in the diet and susceptible teeth (Houte, 1994).

Some syndromes which have chromosomal abnormalities reported to be associated with low caries indices. Down syndrome is an example of this condition (Hennequin *et al.*, 1999) however; the reason of the low incidence of caries in the Down syndrome is unclear (Takashi *et al.*, 2001).

Down syndrome is one of the chromosomal disorders caused by an error in cell division that results in the presence of an additional third chromosome 21 or "trisomy 21" and it is the most frequent genetic cause

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of mild to moderate mental retardation and associated medical problems. It occurs in one out of 800 live births, in all races and economic groups (Cogulu *et al.*, 2006).

In Egypt, it has been reported that the incidence of Down syndrome occurs in 1 per 1000 births (Mokhtar *et al.*, 2003). Oral findings that may be associated with Down syndrome include mouth breathing, open bite, appearance of macro glossia, fissured lips and tongue, angular cheilitis, delayed eruption times, missing and malformed teeth, small roots, crowding and periodontal diseases (Bell *et al.*, 2002).

Theoretically saliva can affect incidence of dental caries in four general ways, firstly as a mechanical cleansing which result in less accumulation of plaque, secondly by reducing enamel solubility by means of calcium, phosphate, and fluoride, thirdly by buffering and neutralizing the acids produced by cariogenic organisms or introduced directly through diet and finally by anti-bacterial activity (Mandel, 1974). Saliva plays an important role in oral health as it maintains the integrity of the oral hard and soft tissues, protects the oral tissue against immunologic bacterial, fungal and viral infections. A critical role in the prevention of dental caries has been documented as saliva controls the equilibrium between demineralization and remineralization in a cariogenic environment (Siqueira *et al.*, 2004). Salivary buffers can reverse the low pH in plaque and allows for oral clearance thus prevent the demineralization of enamel. It has been suggested that in addition to these properties, the flow rate and viscosity of saliva may influence the development of caries as it was noticed that salivary flow rate less than 0.7 ml/minute can increase the risk for tooth destruction (Yarata *et al.*, 1999). Mutans streptococci are the main cariogenic microorganisms present in the oral cavity especially streptococcus mutans and streptococcus sobrinus, these pathogens can colonize the tooth surface and produce acids at a faster speed than the capacity of neutralization of the biofilm in an oral environment below the critical pH value (less than 5.5) which results in the destruction of the tooth enamel (Zussmanetal., 2007).

Secretory immunoglobulin A (SIgA) constitutes the predominant immunoglobulin isotype in saliva, it is considered to be the first line of defense of the host against pathogens which colonize or invade surfaces bathed by external secretions. The main function of SIgA antibodies seems to limit microbial adherence as well as penetration of foreign antigens into the mucosa. Since dental caries and periodontal diseases are associated with indigenous bacteria, salivary IgA induced against streptococcus mutans leads to a reduction in the colonization of this bacterium and consequently to the prevention of dental caries (Humphrey, 2001). Since saliva has many properties that may serve to maintain oral health, create an appropriate ecologic balance and play a great role in dental caries process it was of special interest to search for the possible reasons of low caries incidence in the salivary constituents of Down syndrome children.

Aim of the study:

The aim of this study is to investigate the relationship between various salivary composition and dental caries among a group of Egyptian Down syndrome children.

MATERIALS AND METHODS

A total of 30 Egyptian children were included in this study, their ages ranged from eight to fourteen years. They were divided into two groups; group I includes twenty children with Down syndrome, and group II (control) includes ten apparently healthy children. In the tested group the children were selected from those attending the Outpatient Clinic of the Clinical Genetic Department, Genome Research and Human Genetic Division, National Research Centre, while the control group was selected from the relatives of the test group patients. Collected data have been used for study purpose only. The parents of the children under study were informed about the purpose of the study and of the name of the research institute before agreeing to participate. Assurance was given that their cooperation was voluntary and that no negative consequences would result to those who decided not to participate in the study. Also, the parents were informed that they could skip any question they did not want to answer.

Dental caries was assessed for the presence or absence using the methods described for basic oral health surveys by the world health organization "W.H.O. recommendations 1987" to calculate dental caries index. For each child 5 ml saliva was collected and divided as follows; 1 ml for testing PCR and IgA, 1 ml for testing electrolytes, 2 ml for testing pH and viscosity and 1 ml for culturing of streptococcus mutans bacteria. Measuring the pH and viscosity were done using pH meter (engineering system and design (ESD) pH 5g, USA) and viscometer (Elansari micro viscometer) while measuring the SIgA concentration in Saliva was done by using ELISA kit (Gamma Crade Company, GTCO) and ELISA Machine (Tecon).

As regarding the salivary electrolytes concentration (fluoride, calcium, potassium and phosphorus) it was done by using ion chromatography (Dionex Dx600).

The microbiological examination for streptococcus mutans bacteria was done by culturing using mitis salivarius bacitracin agar [mitis salivarius (BD Difco, France), bacitracin disks, potassium tellurite solution (BBL Difo, France), micropipette (pipetman Gilson, Gilson Medical Electronics, France), Candle Jar (BBC, DA124, du scientific, England) and electronic incubator (Edelstahl, Rost Feri, Germany)].

Also a molecular method for identification of streptococcus mutans bacteria using polymerase chain reaction [AxyPrep Multisource Genomic DNA Miniprep Kit for the purification of genomic DNA (Biomol Egypt, Egyptian Biotechnology Company, Nasr City, Cairo)] was done with the following steps; DNA extraction from saliva, identification of st. mutans by PCR using specific primers [The sequence of the primers was as follows: forward primer (CEFK-F) was common for all of the serotypes and designed within the 3' end region of *rgpE* (5'-ATTCCC GCCGTTGGACCATTCC-3'), whereas the serotype c, e and f specific reverse primer (CEF-R) was designed within the serotype k-specific 5' region of the *rgp-F* (5'-CCGACAAA GACCATTCCATCTC-3')], PCR amplification, PCR products were analyzed by electrophoresis in 1.5% Agarose gel, finally quantitation of gene expression was done using densitometer.

The obtained data statistically analyzed, presented in tables and showed in graphs.

RESULTS AND DISCUSSION

The mean and standard deviation of the five tested salivary parameters in the two groups were statistically computed and analyzed (table,1 and figure 1,2).

Table (1) shows the mean, standard deviation and P value of each variable (DMF, pH, viscosity, IgA, salivary electrolytes and St. Mutans culture count) in normal and Down syndrome groups.

The results of this study show that the DMF was lower in Down syndrome group than in the control children.

Regarding the pH and viscosity values, Down syndrome group has a statistically significant lower means when compared to the control one^s.

Normal children have a higher level of streptococcus mutans count by both culturing and Polymerase chain reaction methods than that in the Down syndrome group.

High level of salivary electrolytes (calcium, phosphorous, potassium and fluoride) and IgA in Down syndrome group was observed than in those of normal children.

The correlation between PCR and IgA with other different variables (DMF, IgA and St. mutans culture count) in both control and Down syndrome groups respectively are presented in Table, 2 and Figure 3 and 4. For the control group children there was a direct correlation between PCR, DMF and St. mutans culture; this correlation was statistically significant ($r = 0.857$ and $r = 0.932$ respectively) and a negative correlation between PCR, IgA was observed ($r = -0.879$). While for the Down syndrome children there was a positive correlation between PCR, DMF and St. mutans culture; this correlation was statistically significant ($r = 0.653$ and $r = 0.559$) and an inverse correlation between PCR and IgA which was not statistically significant ($r = -0.389$).

As regarding the correlation between IgA, DMF and St. mutans culture in normal children, there was a negative correlation between IgA, DMF and St. mutans culture. This correlation was statistically significant ($r = -0.809$, $r = -0.918$). While there was an inverse correlation between IgA, DMF and St. mutans culture in Down syndrome group. This correlation was statistically significant ($r = -0.632$) with DMF and not significant with St. mutans culture ($r = -0.347$).

Table (3) and Figure (5) shows the correlation between DMF and other variables (pH, viscosity, St. mutans culture count, and Ca, P, K, FL salivary electrolyte).

When the pH value was correlated to the DMF, the results of this study showed that there was a statistically significant inverse correlation between them ($r = -0.749$).

There was a positive correlation between DMF and each of viscosity and St. mutans count, this correlation was not statistically significant for viscosity ($r = 0.259$), while for St. mutans count it was statistically significant ($r=0.606$).

Negative correlation between DMF, and the following salivary electrolytes; Calcium, Phosphorous, Potassium and Fluoride respectively ($r = -0.584$, $r = -0.636$, $r = -0.700$ and $r = -0.685$).

In Table (4), the regression coefficients of PCR and St. mutans culture count as a predictor of caries activity reveal that PCR is a significant predictor of caries activity ($P < 0.000$ logistic regression analysis).

Table 1: Means and standard deviation values of the different variables in normal and Down syndrome group.

Group	Normal	Down syndrome	P-value
Variable	Mean± SD	Mean± SD	
DMF	2.0 ± 1.5	0.35 ± 0.7	<0.001*
pH	6.5 ± 0.4	6.84 ± 0.3	0.006*
Viscosity	1.13 ± 0.05	1.06 ± 0.03	0.001*
Calcium	1.72 ± 0.2	1.88 ± 0.2	0.008*
Phosphorous	12.6 ± 1.1	22.66 ± 1.1	<0.001*
Potassium	13.45 ± 1.1	14.73 ± 1	0.007*
Fluoride	0.51 ± 0.4	1.15 ± 0.5	0.001*
St. mutans	7.35 ± 0.05	7.21 ± 0.03	<0.001*
PCR	1530.7 ± 192	931.1 ± 78.7	<0.001*
IgA	681.8 ± 145	1074.8 ± 153.1	<0.001*

SD = standard deviation

*: Significant at $P \leq 0.05$

Table 2: Correlation between both PCR and IgA with different variables.

Group	Normal	P-value	Down syndrome	P-value
Variable	Correlation coefficient		Correlation coefficient	
Correlation between PCR and different variables				
DMF	0.857	0.001*	0.653	0.021*
IgA	-0.879	0.001*	-0.389	0.090
St. mutans	0.932	0.001*	0.559	0.010*
Correlation between IgA and different variables				
DMF	-0.809	0.003*	-0.632	0.003*
St. mutans	-0.918	0.001*	-0.347	0.134

*: Significant at $P \leq 0.05$

Table 3: Correlation between DMF and different variables.

Variables	Correlation coefficient (r)	P-value
pH	-0.749	0.001*
Viscosity	0.259	0.067
Calcium	-0.584	0.001*
Phosphorous	-0.636	0.001*
Potassium	-0.700	0.001*
Fluoride	-0.685	0.001*
St. mutans	0.606	0.001*

*: Significant at $P \leq 0.05$

Table 4: Regression coefficients of PCR and culture as a predictor of caries activity.

Variable	Un standardized coefficients		Standardized coefficients		t-test	P-value
	Beta	Std. Error	Beta			
PCR	0.002	0.000	0.905		6.397	<0.000*
Culture	-0.909	0.592	-0.217		-1.536	0.131

*: Significant at $P \leq 0.05$

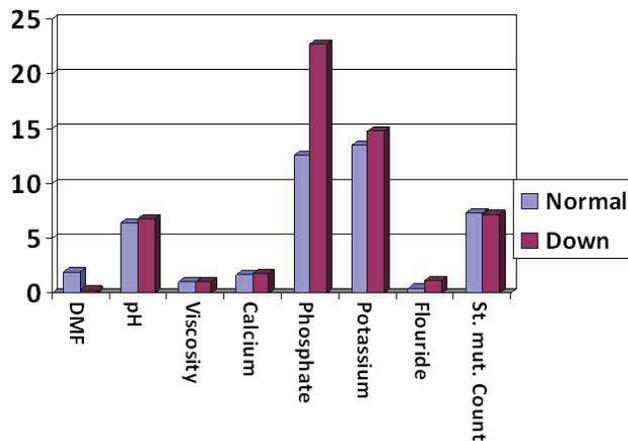


Fig. 1: Mean values of the different variables in normal and Down syndrome group.

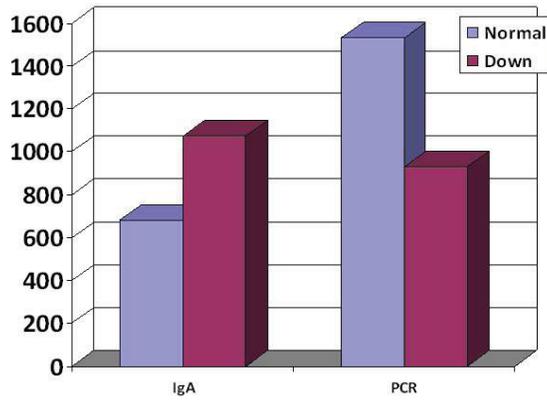


Fig. 2: Mean values of IgA and PCR in normal and Down syndrome group.

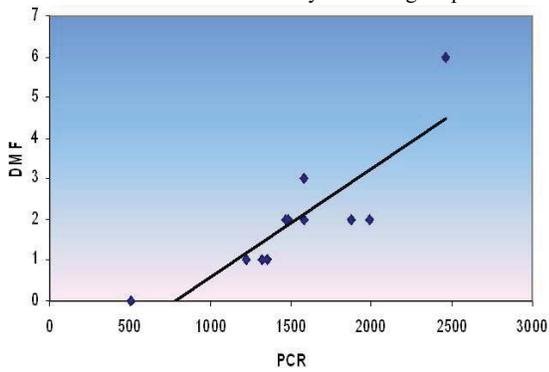


Fig. 3a: Correlation between PCR and DMF in control group

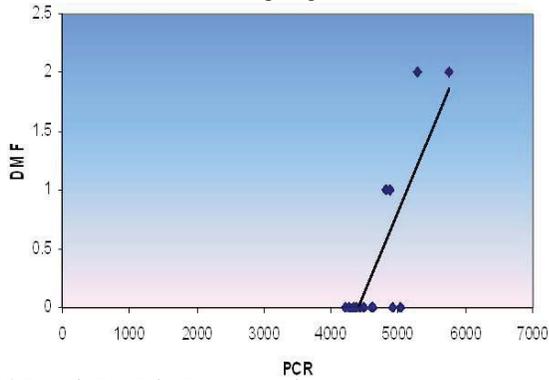


Fig. 3b: Correlation between PCR and DMF in Down syndrome group.

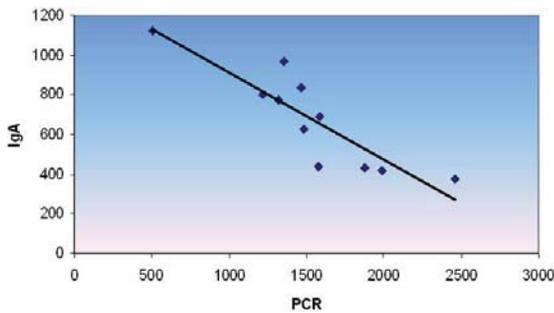


Fig. 3c: Correlation between PCR and IgA in control group

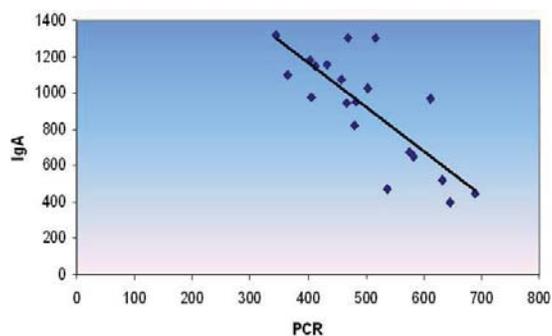


Fig. 3d: Correlation between PCR and IgA in Down syndrome group.

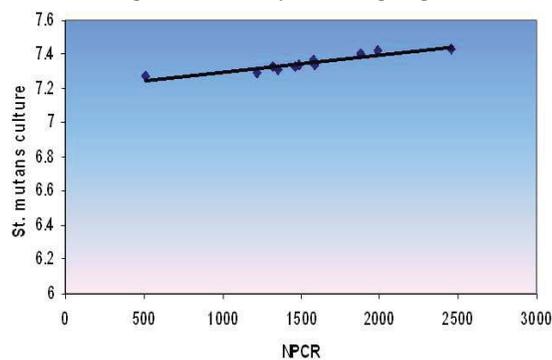


Fig. 3e: Correlation between PCR and st. mutans count in control group

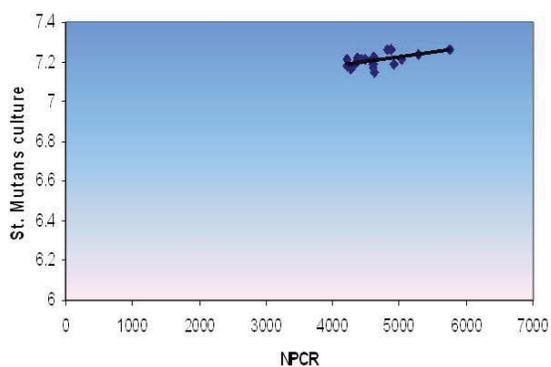


Fig. 3f: Correlation between PCR and st. mutans count in Down syndrome.

Fig. 3: Correlation between PCR and various values for normal and Down syndrome group.

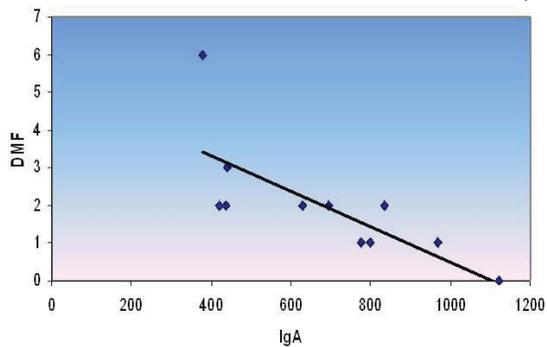


Fig. 4a: Correlation between IgA and DMF in normal group.

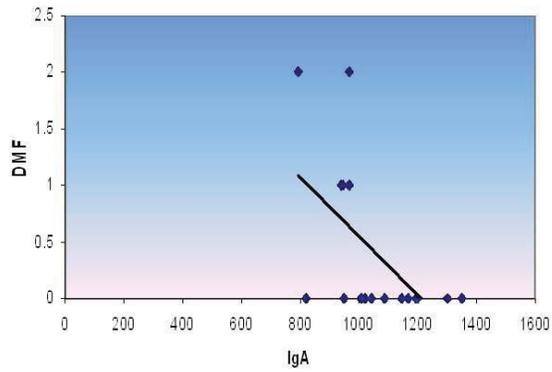


Fig. 4b: Correlation between IgA and DMF in Down syndrome group.

Fig. 4: Correlation between IgA and DMF values for normal and Down syndrome group.

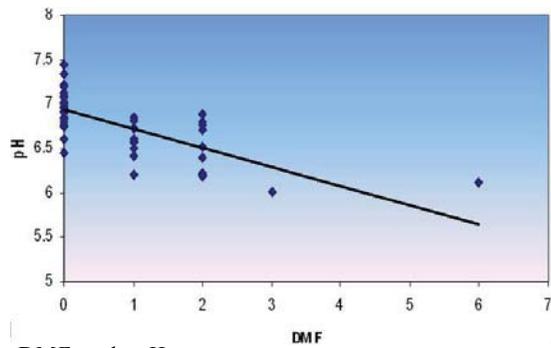


Fig 5a: Correlation between DMF and pH .

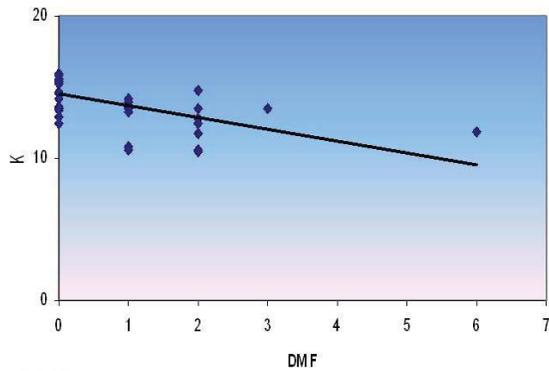


Fig. 5b: Correlation between DMF and Potassium.

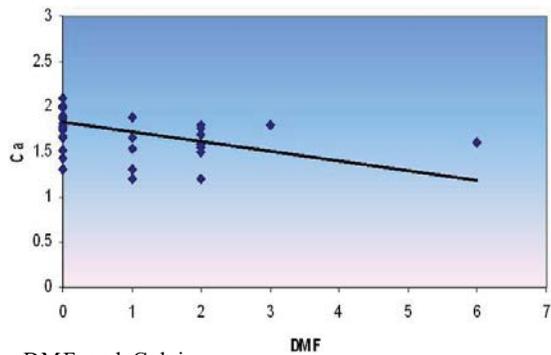


Fig. 5c: Correlation between DMF and Calcium.

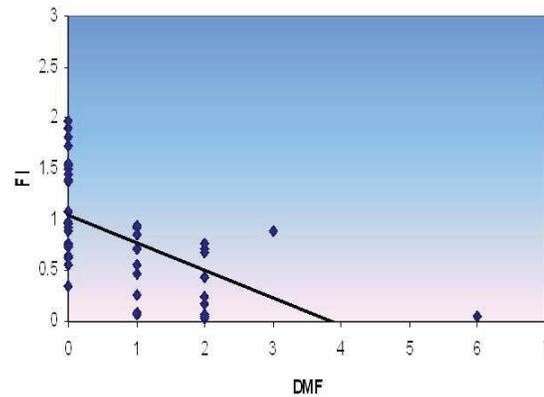


Fig. 5d: Correlation between DMF and Flouride.

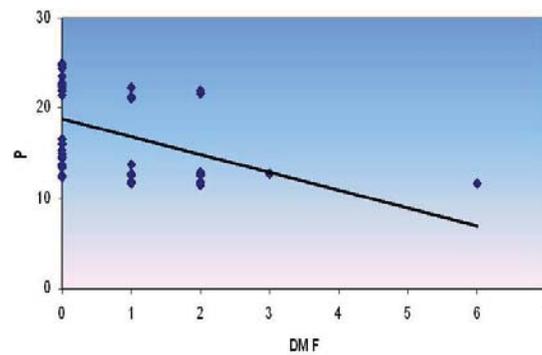


Fig. 5e: Correlation between DMF and Phosphorous.

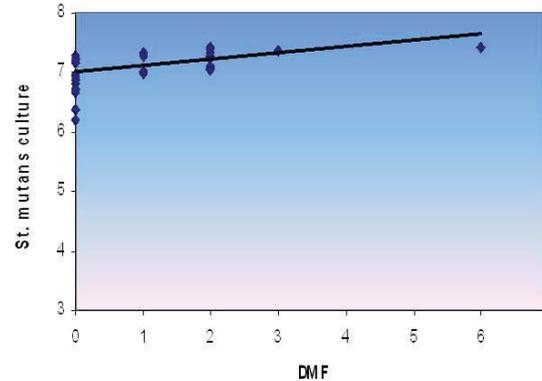


Fig. 5f: Correlation between DMF and st. mutans count.

Fig. 5: Correlation between DMF and various values for Down syndrome group.

Discussion:

There is a general agreement among dental professionals that the salivary secretion and substances secreted within saliva influence to a high degree the strength of individual caries attack, so it has been alleged that salivary composition is an important factor in determining the prevalence of dental caries.

Most studies have suggested that the reduction of dental caries in Down syndrome children than that of normal one's may be explained by congenital oligodontia, delayed eruption, a different salivary composition (salivary IgA, salivary pH, buffering capacity and flow rate) or a difference in eruption times as the teeth of children with Down syndrome often erupts in 1-2 years later than that of the normal child. Often the sequence of eruption of teeth is different than expected (Hennequin *et al.*, 1999). Also sometimes there are missing teeth

(usually the upper incisor) or the teeth are peg-shaped or pointed and sometimes are small. This agrees with many investigators as Lee *et al.*, 2004, Cogulu *et al.*, 2006 and Castilho *et al.*, 2007. However, the precise cause of the lower prevalence of dental caries in Down syndrome children is still unclear.

The decision to collect unstimulated saliva in this study was chosen as unstimulated whole saliva often yields valuable information and usually correlates to clinical conditions more accurately than stimulated saliva, this is coincide with the work of Bardow *et al.*, 2001 and Malamud *et al.*, 2006.

The statistically significant inverse correlation between pH value and DMF coincide with the work of Mandel, 1974 and Zhou *et al.*, 2007 as both reported a higher pH in saliva of persons who are immune from caries than in those who are susceptible. This opinion disagrees with Vitorino *et al.*, 2006 who stated that stimulated salivary pH is positively correlated with DMFT index. This conflict may be due to the difference in method and time of collecting saliva sample as the diurnal pH values differ from nocturnal one.

The positive (not statistically significant) correlation between DMF and viscosity may be explained as it is very difficult to get pure unstimulated saliva, since the imagination of eating food and the concentration upon saliva production will allow a decrease of viscosity and an increase in the speed of secretion. This psychic stimulus of the quantity and quality of the saliva may be the reason why the simple connection between caries susceptibility and viscosity was not recognized. The low values of salivary viscosity of Down syndrome children may be due to the alterations in salivary composition as the viscosity of the whole saliva increase with reduction in pH and calcium level (Biesbrock *et al.*, 1992).

Dental caries constitute an infectious disease caused by bacterial infection, in which *St. mutans* plays an important role. The present investigation revealed that Down's syndrome group has lower level of *Streptococcus mutans* count (by culturing and PCR methods) than that of the control group, this agree with Morinushi *et al.*, 1995 and Szilgyi *et al.*, 2000 who found a direct relationship between the *Streptococcus mutans* and dental caries in patient with Down. The positive correlation between *St. mutans* counts (by culturing and PCR methods) and DMF which found in this study was previously proven by other investigators; Koga- Ito *et al.*, 2004; Law and Seow, 2006 and; Olak *et al.*, 2007.

In this study Logistic regression analysis was done to compare between microbiological examination for *Streptococcus mutans* bacteria (using culture with mitis-salivarius-bacitracin agar media) and molecular methods (using PCR) as predictors of caries activity. The results show that PCR is a significant predictor of caries activity. This result was in agreement with Aguilera *et al.*, 2002 who stated that polymerase chain reaction is a sensitive, specific and non-time consuming method.

The inverse correlation between DMF, and salivary Calcium ion concentration came in accordance with the results of a previous studies of Kedjarune, *et al.*, 1997 and Cornejo *et al.*, 2008 but it is not in accordance with Lenander-Lumikari *et al.*, 1998 who found no correlation with whole stimulated saliva between calcium level and dental caries.

Regarding the correlation between Phosphorous level and DMF, the result showed that there was an inverse correlation between DMF and Phosphorous. Phosphorous has also been reported by many investigators to have caries prevention effects (Shannon, 1964 and Duggal *et al.*, 1991).

The higher salivary Phosphorous and Potassium level in Down syndrome children than those of control group is confirmed by the work of Winer and Feller, 1972 who found an elevation in the Phosphorus and Potassium level in mongoloid patients. This result was disagreed with Siqueira *et al.*, 2004 who found a lower concentration in Potassium level in the Down syndrome group than normal group. When the correlation between the Fluoride level and DMF was assessed, the present study showed that there was an inverse correlation between DMF and Fluoride level, this was found in agreement with the result of John, 2000 and Robinson *et al.*, 2004.

The results of this study showed that Salivary IgA is elevated in Down syndrome group than in normal group. Several studies have shown similar results as the low caries prevalence in Down syndrome children appears to be due to immune protection caused by the elevated salivary IgA concentrations. This may reflect a more established state of immunization in Down syndrome than in the normal subjects (Lee *et al.*, 2004 and Cogulu *et al.*, 2006). It has been suggested that salivary IgA antibodies play an important role in the immune response against dental caries, which are generated by the common mucosal immune system. These antibodies may reduce the initial adherence of the bacteria to saliva-coated teeth surfaces; neutralize extracellular enzymes as well as the possible inhibition of the metabolic activities. The statistically significant inverse correlation between salivary IgA and DMF which was observed in the present study coincide with the work of Bratthall *et al.*, 1997 and Siamopoulou *et al.*, 2000 as they reported that caries-free patients have significantly higher levels of naturally occurring salivary SIgA compared with caries-active subjects.

Finally we can concluded that the low caries index observed in Down syndrome children compared to healthy apparent children is related to the alkaline pH, lower viscosity, lower St. mutans count, higher concentration of salivary electrolytes and the significant rise in immunoglobulin A in their saliva.

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