

# Selected salivary constituents, physical properties and nutritional status in relation to dental caries among 4-5 year's old children (Comparative study)

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## ABSTRACT

**Background:** Tooth decay is still one of most common diseases of childhood, child's primary teeth are important even though they are temporary. This study was conducted to assess the physiochemical characteristic of saliva among caries experience preschool children and compared them with caries free matching in age and gender. Then an evaluation was done about these salivary characteristics to dental caries and evaluated the relation of body mass index to dental caries and to salivary variables.

**Materials and method:** After examination 360 children aged 4-5 years of both gender. Caries-experiences was recorded according to dmfs index by (World Health Organization criteria 1987) during pilot study children with caries experience was divided in to three groups according to decay fraction of decay missing filled surfaces index. Mild with decay surfaces <6, moderate with 6 ≤ decay surfaces ≤ 13 and severe with decay surfaces > 13 and select thirty children with moderate caries experience and compared with thirty caries free children decay missing filled surfaces = 0 match in age and gender. Nutritional status of each child was assessed by measuring weight and height to calculate body mass index. Unstimulated saliva collected from sixty child under standardized condition and potential hydrogen and flow rate were measured. Total antioxidant concentration, total protein, calcium, inorganic phosphorus, zinc and copper were measured.

**Results:** Statistically highly significant differences were found in concentration of salivary calcium and inorganic phosphorus between caries-experience and caries free children with higher mean value among caries free group. Statistically significant differences were found in concentration of salivary zinc between groups. Statistically non-significant differences were found in concentration of salivary total antioxidant, total protein, copper and body mass index between caries experience and caries free groups. Salivary flow rate and PH showed statistical non-significant differences between groups. Positive non-significant correlation was found between dmfs index, (ds) fraction of dmfs index and salivary flow rate, total antioxidant and negative correlation with salivary PH, total protein, calcium, phosphorus, zinc and copper. Positive a non-significant correlation was found between BMI, salivary flow rate, PH, total antioxidant, zinc and copper while a negative weak correlation found with dmfs, ds fraction of index and salivary total protein, calcium and inorganic phosphorus in caries experience group. Furthermore a non-significant positive correlation was found between BMI, salivary flow rate, PH, total antioxidant in addition to that negative weak correlation was found with salivary total protein, calcium inorganic phosphorus, zinc and copper among caries free group.

**Conclusion:** The findings of the present study showed that inorganic components of saliva play an important role in remineralization of incipient caries and there is an inverse association between body mass index and dental caries.

**Keywords:** dental caries, BMI, saliva, inorganic components. (J Bagh Coll Dentistry 2014; 26(2): 150-156).

## INTRODUCTION

Dental caries is one of the most common, communicable and intractable infectious disease in human. It remains the persistent important oral health problem internationally and particularly among developing countries <sup>(1-4)</sup>. Early childhood caries is most common chronic illness among children and adolescent, caries progression can lead to pain and reduced ability to chew and eat which may also lead to iron deficiency due to malnutrition.

Reduction of quality of life for children with early childhood caries resulting from disturbed sleeping and concentration problems <sup>(5)</sup>. Saliva is biological fluid in oral cavity composed of mixture of secretory product from major and minor salivary gland. The fact that teeth are in co-

stant contact with and bathed by saliva suggests this environmental agent would profoundly influence the dental caries process <sup>(6,7)</sup>.

A protective potential role of saliva in pathogenesis of caries process has been implicated in various studies, but possible role of endogenous host-associated attributes of saliva in disease process has so far received little attention <sup>(8)</sup>. Among which one of most important function of saliva is defense function to the specific and nonspecific antibacterial factor included in it as well as to antioxidant defense system, antioxidants have many health benefits that made their evaluation in disease process very popular <sup>(9-12)</sup>.

Relative protections against dental cavities, flow rate, buffering capacity, calcium, phosphate, and fluoride concentration are essential <sup>(13)</sup>. Presences of various types of trace elements in saliva, certain investigators have reported that trace elements to be cariogenic and others as

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cariostatic<sup>(14)</sup>. Saliva contain a large number of proteins that participate in protection of oral tissue in addition several peptides with bacterial killing activity have been identified for instance lysozyme, lactoferrin, immunoglobulin, mucin and histatin<sup>(15)</sup>. The calcium and phosphate ions in saliva will help to prevent dissolution of dental enamel and help remineralization phase, the calcium is most efficient PH buffer for regulating body fluid while phosphates have additional advantage of being resistant to depression of plaque PH towards the critical PH<sup>(16)</sup>. Nutrition is an integral component of oral health, there is continuous synergy between nutrition and integrity of oral cavity in health and disease. Malnutrition may affect the development of oral cavity and progression of oral disease through altered tissue hemostasis, reduced resistance to microbial biofilm and reduced tissue repair capacity<sup>(17,18)</sup>. National study from Sweden conducted by Alm et al<sup>(19)</sup> suggests positive correlation between dental caries and body mass index (BMI). Other shows an inverse relationship<sup>(20)</sup>.

As for literature available very little has been discussed about dental caries and salivary total antioxidants while no previous Iraqi study has been conducted to investigate relation of total antioxidant and inorganic composition of saliva in relation to moderate caries experience among kindergarten children. For these reasons this study was conducted.

## MATERIALS AND METHODS

### Sample

This study include preschool age children of both gender collected from private and governmental kindergarten schools from different region in Baghdad city, the work in this study extend in period from 12 of December 2012 till the end of April 2013. After examination include 360 child age (4-5) years of both gender, selected sixty children and divided in to two groups caries experience group (study group) thirty child, caries experience measured according to dmfs index (ds) fractions and classify children with different caries experience in to three groups during pilot study mild with  $ds < 6$ , moderate with  $6 \leq ds \leq 13$  and severe with  $ds > 13$ <sup>(21)</sup>, and select moderate caries experience group and thirty child caries free (control group) with dmfs zero caries status recorded using<sup>(22)</sup>. Both groups match in age and gender. Any child had erupted lower permanent molar and or incisor was excluded, child on medication, child with systemic or local disease which affect salivary secretion.

### Assessment of nutritional status: Measurement of weight

Children were weighed by bathroom scale, children reading was recording to the nearest of 0.1kg as possible. The instrument used was checked and standardized against a known weight of 5kg and adjusted in the morning before measurements were started and after weighing every 20 children<sup>(23)</sup>. Children were weighed with minimum clothes without shoes and head covering and without touching anything, then 500 gram were subtracted from the total weight to compensate of the light underneath cloths<sup>(24)</sup>.

### Measurement of Height

The height of the child was measured by using ordinary measuring tape fixed at the wall, the child was standing up after removing his/her shoes with feet parallel to each other and pointed forward and the back of the child is straight in upright position. The knee was straight and the child's head was in position that the line between the lower boarder of the orbit and the upper margin of the external auditory meatus (Frankfort plane) is horizontal. The sliding head piece is lowered to rest on the head; the measurement should be recorded to the nearest 0.1cm<sup>(23)</sup>. **Body mass index (BMI):** This index is a number calculated from child's weight and height, according to this formula:

$$BMI = \frac{\text{weight (kg)}^{(25)}}{\text{height}^2 \text{ (m}^2\text{)}}$$

Because of unavailability of Iraqi standard for comparison, the value of nutritional indicators were compared with the international reference values, for this purpose it was recommended to use the reference population that defined by National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion and using CDC growth charts.

### Collection of salivary samples

Collection of saliva is done at morning 9-11 am in the second appointment to reduce the anxiety and fear that might occur after clinical examination unstimulated saliva collected in a sterile graduated test tube. Resting saliva collected for 10 minute by spitting method<sup>(26)</sup>. PH of resting saliva was measure using digital PH meter, the flow rate of saliva was expressed as millilitre per minute (ml / min) after the foam is all disappeared<sup>(27)</sup>. The samples were centrifuged at 1500rpm for 5minute the clear supernatant was separated by micropipette and was stored in a deep freeze for the subsequent analysis which was carried<sup>(28)</sup>.

### Biochemical analysis of saliva

Essential elements of saliva (calcium, zinc and copper) were analysed by using flame atomic absorption spectrophotometer following standardized procedure. While total antioxidant concentration (TAC) was measured by using A ready-made kit (Cayman Chemical Company) and analysed by Enzyme Linked –ImmunoSorbent Assay (ELISA) Machine. Total protein, inorganic phosphorus measured by using A ready-made kits of (Syrbio, Biomaghrheb, Tunisia respectively) and analyzed by UV visible recording spectrophotometry (Cecil CE 7200 UK) machine.

### RESULTS

The results showed that the test distribution is normal for all readings of the studied parameters in the caries free and caries experience groups by using One-Sample Kolmogorov-Smirnov test procedure and that indicating the successfulness of applying the conventional methods either of descriptive or inferential tools of the statistical parametric hypotheses. Sixty children included in this study. Thirty of them were caries experience with (ds) fraction ranging from 6-13 as study group, and other thirty were caries free with dmfs=0 as control group matching in age and gender. age range of children was 4-5 years, 53.6% boys of both group at age 4 years, 46.4% girls of both groups at age 4 years, 50% boys of both groups at age 5 year, 50% girls of both groups at age 5 years.

Table (1) demonstrates comparison between the caries experience and caries free group regarding salivary flow rate measured in (ml/min) and PH. The result showed statistically non-

significant differences at  $p > 0.05$ . Table (2) demonstrate comparison of salivary total antioxidant concentration measured in (mM) and total protein measured in mg/dl the results showed statistically non-significant differences at  $p > 0.05$ . Statistically highly significant differences at  $p < 0.01$  between caries experience and caries free regarding salivary calcium and inorganic phosphorus with higher value among caries free group. The results showed that the caries free group had higher mean value than the caries experience group ( $5.86 \pm 1.77$ ,  $4.90 \pm 1.22$  respectively) with statistical significant differences at  $p < 0.05$  in concentration of salivary zinc table (4). Statistically non-significant differences at  $p > 0.05$  in concentration of salivary copper and BMI between caries experience and caries free group table (5). A non-significant positive weak correlation found between dental caries and salivary flow rate and TAC while a non-significant negative weak correlation found between dental caries and salivary PH, total protein, phosphorus, zinc, copper and calcium. A non-significant positive weak correlation found between BMI, salivary flow rate, PH, total antioxidant, zinc and copper. A non-significant negative weak correlation found with dental caries and salivary total protein, calcium and phosphorus among caries experience group. A non-significant positive weak correlation found between body mass index and salivary PH, flow rate, total antioxidant. A non-significant negative weak correlation found with salivary total protein, calcium, phosphorus, zinc and copper among caries free group.

**Table 1: Comparison of salivary flow rate ml/min PH between caries experience and caries free groups**

Variables	No.	Caries experience		Caries free		d.f.	t-test	P-value
		Mean	SD	Mean	SD			
Flow rate	30	0.42	0.24	0.38	0.23	58	-0.77	0.44
PH	30	6.94	0.33	6.98	0.37	58	-0.35	0.72

**Table 2: Comparison of salivary TAC (mM) and total protein mg/dl between caries experience and caries free groups**

Variables	No.	Caries experience		Caries free		d.f.	t-test	P-value
		Mean	SD	Mean	SD			
TAC	30	0.22	0.18	0.20	0.15	58	-0.48	0.63
Total protein	30	422.00	51.17	438.30	75.68	58	0.98	0.33

**Table 3: Comparison of salivary calcium and phosphorus mg/dl between caries experience and caries free groups**

Variables	No.	Caries experience		Caries free		d.f.	t-test	P-value
		Mean	SD	Mean	SD			
Calcium	30	3.09	0.67	4.52	0.72	58	7.95	0.000
Phosphorus	30	3.83	2.30	6.97	2.85	58	4.70	0.000

**Table 4: Comparison of salivary zinc  $\mu\text{g}/\text{dl}$  and between caries experience and caries free groups**

Variables	No.	Caries experience		Caries free		d.f.	t-test	P-value
		Mean	SD	Mean	SD			
Zinc	30	4.90	1.77	5.86	1.22	58	2.45	0.017

**Table 5: Comparison of salivary copper  $\mu\text{g}/\text{dl}$  and BMI between caries experience and caries free groups**

Variables	No.	Caries experience		Caries free		d.f.	t-test	P-value
		Mean	SD	Mean	SD			
Copper	30	3.73	1.20	3.35	1.52	58	-1.08	0.28
BMI	30	16.38	2.01	16.40	2.12	58	0.05	0.96

## DISCUSSION

Dental caries a common disease in children, if untreated can affect body weight, growth and quality of life in preschool children<sup>(29)</sup>. In young children as in adult caries can begin as soon as the teeth erupt and can rapidly progress to extensive decay of all primary teeth<sup>(30)</sup>. Decision was made to collect unstimulated whole saliva because this type of saliva predominates during most part of the day and is important for maintenance of oral health, reflecting the physiological status of the oral cavity and the entire body<sup>(31)</sup>. Under resting conditions without the exogenous stimulation that is linked with feeding there is a slow flow of saliva which keeps the mouth moist and lubricates the mucous membrane. This unstimulated flow which is secreted by the salivary glands majority of the time. Unstimulated saliva is essential for the health and well-being of the oral cavity and also provides a strong protective effect to the oral cavity, against dental caries<sup>(32, 33)</sup>. The results of the present research found non-significant differences attributed to salivary flow rate located within normal range. Unstimulated flow rates of less than 0.1 mL/minute are considered evidence of hyposalivation, The unstimulated flow rate averages 0.3 to 0.4 milliliter per minute, but the range is wide<sup>(34)</sup>. and higher mean value with study group and positive correlation with dental caries could be attributing to moderate caries experience, dmfs index is a life-time cumulative index of dental disease and treatment and may have little bearing on caries activity at a specific point in time and one-time determination of salivary flow rate may not be a comprehensive evaluation of salivary function<sup>(35)</sup>. Finding of the present study can be explained by that salivary PH in subject with low or no caries activity had resting salivary PH of around 7.0 and those with extreme caries activity had resting PH below critical PH (5.5) and also could attributed to the level of pH in the enamel dissolution in caries process which needs to fall below the critical pH<sup>(36,37)</sup>. The data of the present study illustrated a non-significant positive weak correlation between

TAC and dental caries. Such positive correlation although not reach to significance value may demonstrate to that the levels of antioxidants could be altered in response to an infection or disease. The absence of an infectious challenge in the form of caries or poor oral hygiene in the present study among caries free could be one of the factors for the comparatively decreased levels of TAC of saliva<sup>(38)</sup>. Total protein in the present study showed a higher mean level with caries free group than caries experience ( $422.0 \pm 51.17$ ,  $438.3 \pm 75.68$  respectively) with non-significant differences and non-significant weak invers correlation of total protein with dental caries. Result of the present although not reach to statistical significance could related to action of salivary protein such as action of some protein as antimicrobial and PH modulating<sup>(39)</sup> also attributed to function of certain protein like early pellicle protein, proline-rich protein and statherin which promote remineralization of enamel by attracting calcium ion and demineralization process is retarded by pellicle protein in concert with calcium and phosphate ions in saliva and in plaque fluid and several salivary (glycol)protein prevent adherence of oral microorganism to enamel pellicle and inhibit their growth<sup>(33)</sup>.

The finding of the present research interpreted as the saliva which is supersaturated with calcium and phosphate acts as a reservoir for these essential ions. In such a conducive environment the process of remineralization overrides demineralization<sup>(40)</sup>. Calcium in saliva acts as chief mineral to prevent dissolution of teeth via its solubility constant and continuous supply to affected areas of teeth, optimum concentration of calcium in saliva prevents dental caries and promotes remineralization, by giving strength and perfectness to the structure of teeth<sup>(41,42)</sup>. The increase level of phosphorus among caries free give idea about potential effect of inorganic phosphate in remineralization of incipient caries lesion. Inverse relation with ds fraction and dmfs related to its action in buffer and remineralization<sup>(43)</sup> and phosphate can probably

interfere with adherence of pellicle and bacterial plaque to enamel surfaces<sup>(44)</sup>. High level of zinc leads to greater mineralization and accumulation of zinc quantities on surface enamel that becomes more caries resistance<sup>(45)</sup>. Deficiency of micronutrient like zinc can influence amount and composition of saliva and reduce protective effect of saliva<sup>(46)</sup>. Increased susceptibility to dental caries in zinc-deficient animals might be mediated by alterations in salivary proteins that are associated with the maintenance of tooth structure<sup>(47)</sup>. The inverse relation of copper with dmfs index and ds could be related to the divalent metal ions properly inhibit glycolysis in dental biofilm and anti-biofilm effect related to antimicrobial activity and displacement of  $Ca^{++}$  ions from pellicle and microbial surface and change of microorganism adherence<sup>(43)</sup>. These inverse relation of current study between BMI and dental caries although not reach to statistical differences could be explained by under nutrient and deficiencies of specific nutrient do influence the development of teeth and formation and function and secretion of saliva which in turn influence susceptibility to dental caries<sup>(48)</sup> also malnutrition can indirectly increase susceptibility to dental caries by affecting structure of tooth maturation and provide more cariogenic environmental niche and less protective enamel that include hypomineralization<sup>(49,50)</sup>. Furthermore non-significant positive weak correlation obtained between BMI and both salivary flow rate and PH such correlation may be related to the fact that nutritional deficiencies have been found to affect both salivary gland formation and function as well as composition in which moderate to severe protein malnutrition revealed decrease in stimulated secretion rate, lower content of calcium and chloride ions and total protein secretion and impaired immunological and agglutination defense factors of unstimulated saliva<sup>(51)</sup>. There was no previous study concerning correlation of salivary constituents to BMI so the finding could attribute in comparison to serum constituent and discuss correlation systemically, as saliva consider a mirror of serum<sup>(52)</sup>.

The finding in present study related to TAC showed non-significant positive weak correlation with BMI in both caries experience and caries free group. Such positive correlation although not reach to statistical significant point could be attributed to increase BMI, associated with oxidant stress increase. Possible mechanism contributing to the obesity-associated oxidant stress include increase oxygen consumption and subsequent radical production via mitochondrial respiration, increased fat deposition and cell

injury causing increased rate of radical formation<sup>(53)</sup> these finding in agreement with Sfar et al<sup>(54)</sup> who found activity of super oxide dismutase increase in association with increase BMI and consider obesity independent risk factor of free radical production result in an increased antioxidant response. These findings of current study also agree with Keaney et al<sup>(55)</sup> who found strong association between BMI and markers of oxidant stress related to adiposity as main factor for increase oxidant stress. The possible causes for inverse relation of calcium and BMI could be high calcium intake depresses 1,25 dihydroxy vitamin D leading to decrease in intercellular calcium, thereby inhibit lipogenesis and stimulate lipolysis so higher fat oxidation gain by creating a balance of lipolysis over lipogenesis in adipocyte<sup>(56)</sup>. However negative correlation of phosphorus may be related to the role of phosphorus in degradation of fat as fat transport to their many destinations in body through phosphorylation<sup>(57)</sup>. The findings of the current study attributed systemically as there was no previous study concerning relation of salivary zinc with body mass index and such positive correlation found between salivary zinc and BMI could be attributed to role of zinc systemically in that it constituent nearly of 100 enzymes involved in metabolic process and its deficiency causes loss of appetite and poor growth<sup>(58)</sup>.

Further more positive correlation of copper with BMI explained by role of copper in proper iron absorption and utilization and deficiency associated with iron deficiency anemia<sup>(59)</sup>.

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