

Salivary cortisol among low birth weight 5 years old kindergarten children in relation to dental caries (comparative study)

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ABSTRACT

Background: Birth weight is a powerful predictor of infant growth and survival. Evidence now shows that children born with low birth weight face an increased risk of chronic diseases and have many health problems including oral health. The aims of this study were to assess the salivary flow rate, viscosity, and salivary cortisol among low birth weight kindergarten children aged 5 years old in Hilla centre, in relation to dental caries and compares them with the normal birth weight children of the same age and gender.

Materials and methods: The total sample involved 80 children (40 low birth weights and 40 normal birth weights) aged 5 years old. The diagnosis and recording of severity of dental caries was recorded through the application of $d_{1-4}mfs$ index according to the criteria described by Mühlemann (1976). The stimulated saliva was collected from the total sample under standardized conditions and then analyzed for measuring salivary flow rate and viscosity, in addition to estimation of salivary cortisol by special cortisol kit using VIDAS® Cortisol S.

Results: The mean rank of $dmfs$, ds , ms and fs were found to be higher among low birth weight than normal birth weight groups, with a statistically significant difference for $dmfs$, ds ($P < 0.05$), highly significant difference for ms ($P < 0.01$) and non significant difference for fs ($P > 0.05$). Concerning the ds grade, data analysis showed a significant difference only for d_1 grade ($P < 0.05$). Salivary analysis demonstrated that the mean rank of salivary flow rate was found to be lower among the low birth weight than the normal birth weight groups with non significant difference ($P > 0.05$). The viscosity of saliva was found to be highly significantly higher among low birth weight than normal birth weight groups ($P < 0.01$). Concerning salivary cortisol, data analysis showed that the mean rank was higher among low birth weight than normal birth weight groups. However, the difference was not significant ($P > 0.05$).

Conclusion: The results of the current research revealed that low birth weight status affect oral health conditions.

Key words: Low birth weight, dental caries, salivary flow rate, salivary viscosity, salivary cortisol. (J Bagh Coll Dentistry 2013; 25(4):126-133).

الخلاصة

خلفية: وزن الولادة يشكل مؤشراً قوياً لنمو الرضع والبقاء على قيد الحياة. الدلائل تشير الآن أن الأطفال الذين يولدون مع انخفاض الوزن عند الولادة يواجهون خطراً متزايداً من الأمراض المزمنة ولها العديد من المشاكل الصحية بما في ذلك صحة الفم. وكانت أهداف هذه الدراسة تقييم معدل تدفق اللعاب، واللزوجة، والكورتيزول اللعابي بين انخفاض الوزن عند الولادة لرياض الأطفال الذين تتراوح أعمارهم بين 5 سنوات من العمر في مركز الحلة، وعلاقته بتسوس الأسنان ومقارنتها مع الوزن الطبيعي عند الولادة للأطفال من نفس العمر والجنس.

المواد والطرق: تضمنت العينة الكلية 80 طفلاً (40 انخفاض الوزن عند الولادة و 40 الوزن الطبيعي عند الولادة) الذين تتراوح أعمارهم بين 5 سنوات. وتم تسجيل شدة تسوس الأسنان من (1976) Mühlemann وفقاً للمعايير التي وصفها $d_{1-4}mfs$. وخلال تطبيق مؤشر وقد تم جمع اللعاب المحفز من العينة الكلية في ظل ظروف موحدة ومن ثم تحليلها لقياس معدل تدفق اللعاب واللزوجة، بالإضافة إلى تحليل الكورتيزول اللعابي باستخدام كت خاص بالكورتيزول.

النتائج: رتبة متوسط تسوس السطوح للأسنان اللبينية، أظهر تحليل البيانات فرقا معنوياً فقط للرتبة ds وفيما يتعلق fs وفرق غير معنوي فيما يخص ms وفرق معنوي كبير للعناية فيما يخص ds , $dmfs$ وجود فرق دال إحصائياً. أظهر تحليل اللعاب أن رتبة متوسط معدل تدفق اللعاب وجدت لتكون أقل بين مجموعة انخفاض الوزن عند الولادة من مجموعة الوزن الطبيعي عند الولادة مع وجود فرق غير معنوي. لزوجة اللعاب وجدت لتكون أعلى بكثير مع فارق معنوي كبير للعناية بين مجموعة انخفاض الوزن عند الولادة من مجموعة الوزن الطبيعي عند الولادة. وفيما يتعلق بالكورتيزول اللعابي، أظهر تحليل البيانات أن رتبة متوسط الكورتيزول أعلى بين مجموعة انخفاض الوزن عند الولادة من مجموعة الوزن الطبيعي عند الولادة. ومع ذلك، كان الفارق غير معنوي.

الاستنتاج: كشفت نتائج البحث الحالي أن حالة انخفاض الوزن عند الولادة تؤثر على الأوضاع الصحية للفم.

الكلمات الرئيسية: انخفاض الوزن عند الولادة، تسوس الأسنان، معدل تدفق اللعاب، لزوجة اللعاب، الكورتيزول اللعابي.

INTRODUCTION

Low birth weight (LBW) is a major determinant of mortality, morbidity and disability in infancy and childhood and also has a long-term impact on health outcomes in adult life. The consequences of poor nutritional status and inadequate nutritional intake for women during pregnancy not only directly affect women's health status, but may also have a negative impact on birth weight and early development. Low birth weight also results in substantial costs to the health sector and imposes a significant burden on society as a whole (1).

Low birth weight defines a heterogeneous group of infants: some are born early, some are born growth restricted, and others are born both early and growth restricted. It is generally recognized that being born with low birth weight is a disadvantage for the baby (2). Some authors defined low birth weight as baby weighing less than or equal to (2500 g) (3,4) while others defined LBW as a baby that weighs less than (2500 g) (up to and including 2499 g) regardless of gestational age (5,6).

Children with low birth weight are at risk for medical problems in childhood and adulthood and often have poor oral health. Dental caries remain one of the major global public health problems. It is considered as multifactorial since it is influenced by dietary as well as host factors (7).

However, the literatures contain contradictory

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results concerning the relation between LBW and dental caries. Saraiva et al.⁽⁸⁾, Javadinejad et al.⁽⁹⁾, and Rajshekar and Laxminarayan⁽¹⁰⁾, reported that children born with LBW have higher caries experience in the primary dentition, whereas Gravina et al.⁽¹¹⁾, and Cruvinel et al.⁽¹²⁾, found a greater prevalence of carious lesions in children born at full term than in children born prematurely. They suggested that low birth weight, including preterm births, predisposes to high levels of streptococcal colonization due to reduced immunofunction, in addition that the LBW favoring the development of enamel hypoplasia and salivary disorders^(13,14). In contrast, Burt and Pai⁽¹⁵⁾, Peres et al.⁽¹⁶⁾, and Shulman et al.⁽¹⁷⁾, concluded that no significant association is observed between low birth weight and dental caries.

Saliva is an ideal translational research tool and diagnostic medium as saliva is mirror of the serum and is being used in novel ways to provide molecular biomarkers for a variety of oral and systemic diseases and conditions⁽¹⁸⁻²⁰⁾. Saliva contains various microbes and host biological components that could be used for caries risk assessment⁽²¹⁾.

Saliva represents the first line of defense against dental caries after tooth eruption^(22,23). In addition, saliva displays physical properties related to the maintenance of a healthy oral environment, including secretion rate and viscosity⁽²⁴⁾. This finding tends to support the hypothesis that the mechanism behind the increased risk of dental pathology in preterm, low birth weight and retarded children is centered at structural and functional immaturity of salivary gland which can lead to hyposalivation and low salivary flow in addition to reducing water content in the saliva and thus increasing salivary viscosity^(25,26,27).

Pre- and peri-natal risk factors such as low birth weight and prematurity are presumed to shape the developing brain and predict the development of stress-related problems. In premature infants, a number of complications are considered to be associated with adrenal insufficiency and cortisol values have been examined by a number of investigators in an attempt to identify infants with relative adrenal insufficiency⁽²⁸⁻³⁰⁾. Cortisol is produced in response to stress and is easily measured in saliva⁽³¹⁾.

There have been relatively few studies that have examined the impact of pre- and peri-natal risk factors on salivary cortisol level. Bettendorf et al.⁽³²⁾ found that healthy full-term neonates exhibited higher salivary cortisol concentrations

than healthy preterm neonates. While Wüst et al.⁽³³⁾ reported that birth weight was associated with salivary cortisol responses to psychosocial stress in adult life. Low birth weight predicted higher cortisol reactivity in young adulthood. However, the development of cortisol circadian rhythm in premature infants is not necessarily retarded and that low gestational age is not critical for the timing of emergence of the hypothalamus-pituitary-adrenal (HPA) axis circadian activity⁽³⁴⁾.

Grunau et al.⁽³⁵⁾ reported that extremely low gestational age (ELGA) infants show significantly higher salivary cortisol levels many months past their expected date of delivery, suggesting possible (re-setting) of basal cortisol levels, and long term (programming) of the HPA axis. On the other hand, Schmidt et al.⁽³⁶⁾ concluded that ELBW adults exhibited not significantly lower salivary cortisol at baseline compared with adults born at normal birth weight.

Cortisol is capable of affecting local, mucosal immunity and oral microbial flora, and that mucosal immune competence affects bacterial colonization and growth⁽³⁷⁾. It affects the performance of some immune system cells⁽³⁸⁾. Basal salivary cortisol and cariogenic bacteria were the strongest predictors of dental caries, and from a theoretical perspective, salivary cortisol could plausibly suppress mucosal immunity against cariogenic bacteria⁽³⁹⁾.

The present study was conducted among a group of kindergarten children with low birth weight aged 5 years old in comparison to control group to evaluate the following variables: the occurrence and severity of dental caries, the changes in the physicochemical characteristics of stimulated saliva and these including (salivary flow rate, salivary viscosity, salivary cortisol), in addition to determine the relation between these variables.

MATERIALS AND METHODS

In the present investigation, the study group included 40 children with low birth weight aged 5 years old of both gender. They were examined in their kindergarten during the period from the third of December 2012 till the end of January 2013. The control group included 40 children with normal birth weight who possess as much similarity as possible to the study group with regard to age, gender, social structure and geographic position except in birth weight condition. Both study and control groups should not have any systemic disease that could affect on the salivary analysis. The assessment and recording of caries experiences were done through

the application of decayed (d), missing (m) and filled (f) surface index (dmfs) index for primary teeth. In this study, the decayed fraction of the index was recorded according to the lesion severity using the criteria described by Mühlemann⁽⁴⁰⁾.

The collection of the stimulated salivary samples from the children was performed under standardized conditions according to the instructions cited by Tenovuo and Lagerlöf⁽⁴¹⁾ and Farsi⁽⁴²⁾. Immediately after saliva collection, salivary flow rate was measured freshly and without centrifuged (after foam had all disappeared) by dividing the volume of the collected stimulated saliva in milliliter (ml) on the collection time in minute (min)⁽²⁵⁾. Salivary viscosity was determined by measuring the volume rate of flow through a tube of known dimensions. This was done by using Ostwald's viscometer which is simple device for measuring the viscosity of liquid⁽⁴³⁾. In this study, salivary viscosity was measured at room temperature and before centrifuging of salivary samples⁽⁴⁴⁾. The viscosity of the saliva is measured according to a liquid which have a known coefficient of viscosity, and usually the distilled water is used for this purpose. The flow times of two liquids (saliva and distilled water) which have equal volumes passing through a capillary of the same viscometer are measured and the coefficient of the viscosity of the saliva is determined. The salivary samples were then taken to the laboratory for biochemical analysis and centrifuged at 3000 r.p.m. for 10 minutes. The clear supernatant was separated by micropipette and was stored at (-20°C) in a deep freeze and further assessment for cortisol level in saliva was done by special cortisol kit (VIDAS[®] Cortisol S) using MiniVIDAS technique which is multiparametric immunoassay system⁽⁴⁵⁾. At the end of the assay, results are automatically calculated by the instrument in relation to the calibration curve. These results were obtained in traditional unit (ng/ml). To be converted from traditional to SI units, they had been converted to (nmol/L) by multiplying them by Endocrinology Conversion Factor (2.759)⁽⁴⁶⁾.

Intra and inter calibration were performed to overcome any problem that could be faced during the research, and to ensure proper application of diagnostic criteria used in recording dental status through inter calibration. Statistical Analysis and processing of the data were carried out using SPSS version 19. After exploring the data, it had been found that they were not normally distributed. The non-parametric Mann-Whitney U test was utilized for the parameters of the data

which were not normally distributed and in this test the median and mean rank were used to analyze and determine the differences between the study and control groups. The correlation coefficient tests between the variables were done by using Pearson correlation. The confidence level was accepted at the level of less than or equal to 5%. The highly confidence level was accepted at the level of less than or equal to 1%.

RESULTS

The distribution of the low birth weight and their matching normal birth weight children by gender are shown in Table (1). Dental caries experience in primary dentition represented by dmfs and its components (ds,ms,fs) among low and normal birth weight groups are illustrated in Table (2). Results revealed that the mean rank of dmfs for the total sample was found to be higher among low birth weight than normal birth weight and the difference was significant (Mann Whitney=566.000, Z=-2.280, P=0.023). The mean rank of decay fraction (ds), filling (fs) and missing (ms) surface were found to be higher among low birth weight than normal birth weight with significant difference for ds (Mann Whitney=586.000, Z=-2.087, P=0.037) and highly significant difference for ms (Mann Whitney=625.000, Z=-2.622, P=0.009) while for fs, the difference was not significant (P> 0.05). The grades of decay fraction among low and normal birth weight groups are represented in Table (3). Concerning low birth weight, the d₁ fraction of severity was the highest, followed by d₃, d₂. While the d₄ showed the least fraction of decay severity. For normal birth weight group, the d₄ fraction of severity was the highest, followed by d₂ and d₃. While the d₁ showed the least fraction of decay severity. For the total sample, data analysis showed that the mean rank of d₁ was higher among low birth weight than normal birth weight with statistically significant difference (Mann Whitney=616.000, Z=-2.193, P=0.028). However, the same picture was observed for d₂, d₃, and d₄ with statistically non significant difference (P> 0.05).

The physicochemical characteristics of the stimulated whole salivary flow rate among low and normal birth weight groups are illustrated in Table (4). The mean rank of salivary flow rate was found to be lower among low birth weight than normal birth weight with no significant difference (P> 0.05). Apposite finding was found concerning salivary viscosity as the mean rank was highly significantly higher among low birth weight group than normal birth weight group (Mann Whitney=387.500, Z=-3.970, P=0.000).

The same result was found for salivary cortisol with no significant difference ($P > 0.05$).

Table (5) shows the correlation coefficient between caries experience in primary dentition represented by dmfs and ds components and salivary variables among low and normal birth weight groups. For low birth weight group, the relation between salivary flow rate and caries experience was weak and not significant in positive direction concerning dmfs while in negative direction concerning ds. The same relations were found between salivary viscosity and both dmfs and ds in positive direction while the relations between salivary cortisol and both dmfs and ds were found to be weak, and non significant in negative direction. Concerning normal birth weight, the relations between salivary flow rate and both dmfs and ds were weak and non significant in positive direction. The same relations were found between salivary cortisol and both dmfs and ds while the relations between salivary viscosity and both dmfs and ds were found to be weak, and non significant in negative direction.

Table (6) represents the correlation coefficient between salivary cortisol and both salivary flow rate and salivary viscosity among low and normal birth weight groups. Concerning low and normal birth weight groups, data analysis for the total sample showed that the relation between salivary flow rate and cortisol was found to be weak and non significant in negative direction, while the relation between salivary viscosity and cortisol was found to be weak and non significant in positive direction.

DISCUSSION

Infants born preterm and with low birth weight are at greater risk for mortality, disability and a variety of health and developmental problems compared with infants born at term^(47,48). A fetal programming hypothesis proposed that stressful events during fetal development may program the developing brain for how it handles subsequent stress⁽⁴⁹⁾.

In terms of caries experience among the low birth weight group, the present study showed that the mean rank of dmfs and decay fraction ds were significantly higher than the normal birth weight group. This result was in agreement with the results reported by many previous studies^(8-10,50,51), and was in disagreement with the results that concluded by others^(11,12,52-55).

These results might be due to neonatal malnutrition which increases the risk of poor pregnancy outcomes including low birth weight infants. The greater systemic infant malnutrition

associated with low birth weight children is said to result in systemic insults to the developing primary teeth which can lead to disturbances in the mineralization resulting in hypoplasia^(13,56) and thus predisposing the teeth to caries^(14,57).

Other causes can be attributed to maternal lactation failure, so that lack of breast feeding or exclusive breast feeding for shorter duration in these children leads to undernourishment during maturation phase of teeth and this can lead to hypoplasia and enamel defects which are more susceptible areas to dental caries⁽⁵⁸⁾.

The higher caries experience could be attributed to the lower social class of those children with low birth weight as reported by some previous studies^(59,60). This can be attributed to the low family income and the degree of education in the lower social classes which can affect food selection, nutrient intake, health values, life style, oral hygiene practices, access to health care information and susceptibility to childhood infections^(17,52).

The lower flow rate that was found in the present study (although not significant) among low birth weight children could give another explanation for high caries experience. This can be explained by the fact that many evidences suggest that intrauterine growth restriction (IUGR) leads to impaired growth and maturation of the salivary gland (structural immaturity and lack of differentiated parenchymal elements of the organ may form the basis of its secretory function's lesion) which causes hyposalivation and low salivary flow. This is in agreement with previous study⁽²⁷⁾. Although, dental decay is a multifactorial disease, the salivation's insufficiency is believed to have harmful effect on oral health, this is also shown in the present study as data analysis showed that the correlation between the salivary flow rate and decay fraction (ds) and grades of dental caries (d_{1-4}) were found to be inverse relations among the low birth weight children. This could be attributed to the fact that the higher the flow rate, the faster the clearance, the higher the buffer capacity and the lower dental caries^(61,62).

Increased dental caries might be due to increased saliva viscosity as data of the current study showed high viscosity among low birth weight children. This can be explained by the fact that preterm and low birth weight children are affected by structural and functional immaturity of the salivary gland. This gives reasons for increased viscosity that may be due to reduced water content in the saliva. This finding is in correlation with other study⁽⁶³⁾ and this is also found by data of the present study that showed an

inverse relation exists between salivary viscosity and salivary flow rate which was in agreement with other study⁽²⁵⁾. Increased salivary viscosity plays a role in increasing caries incidence. Among low birth weight children, data analysis of the present study revealed that the correlation of the salivary viscosity with dental caries was found to be a positive relation, which could give another explanation for high caries experience in present study as well as in previous studies⁽⁶⁴⁻⁶⁶⁾ among other study groups. This may be attributed to the fact that saliva that is more viscid is less effective in clearing the mouth from food debris, reduced bacterial co-aggregation, and played a role in increasing caries incidence⁽⁶⁵⁾.

Other causes for high caries experience could be attributed to high salivary cortisol level among low birth weight group (although not significant) and this was in agreement with previous study⁽³³⁾ and can be explained as the higher cortisol level may suppress the oral immunity and induce the proliferation of the cariogenic bacteria leading to increased incidence of dental caries. Basal salivary cortisol secretion was positively associated with dental caries. From a theoretical perspective, salivary cortisol could suppress mucosal immunity against cariogenic bacteria. Basal salivary cortisol secretion and cariogenic bacteria bore strong and independent associations with counts of dental caries⁽³⁹⁾.

Concerning the correlation of the salivary cortisol with flow rate, data analysis of the present study reported an inverse correlation among both low and normal birth weight groups. This is in agreement with some previous studies^(67,68) while disagrees with others^(69,70) among other study groups. This may be explained by the fact that as the flow rate increased, the pH increased but the protein level decreased⁽⁶⁸⁾ and as cortisol is a salivary protein, therefore; its level may be decreased.

The correlation of the salivary cortisol with viscosity was found to be a positive relation in both low and normal birth weight children. The explanation for this can be attributed to the fact that the salivary cortisol is one of the important protein component of human saliva and increasing its value leads to increase viscosity of saliva as viscosity was correlated positively with the total protein concentration^(25,71).

In conclusion, dental professionals need to have comprehensive knowledge about the low birth weight status among children to assess the effects of this status with respect to oral diseases and dental treatment. A comprehensive health care programme including awareness, access to oral health care services, and education regarding

low birth weight status to parents, physicians, nutritionists and pediatric dentists would be beneficial to improve oral and physical health of these children. Further studies incorporating a larger sample size and longitudinal evaluation of the obtained results are warranted to confirm the results of this study.

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Table 1: Distribution of the low birth weight and their matching normal birth weight children according to gender

Gender	Low birth weight		Normal birth weight	
	No.	%	No.	%
Males	21	52.5	21	52.5
Females	19	47.5	19	47.5
Total	40	100	40	100

Table 2: Dental caries experience (dmfs) and components (ds, ms and fs) among low birth weight and normal birth weight children

	Low Birth Weight			Normal Birth Weight			Statistical differences			
	No.	Median	Mean Rank	No.	Median	Mean Rank	df	U-Test	Z- value	P-value
dmfs	40	6	46.35	40	2.5	34.65	78	566	-2.280*	0.023
ds	40	4	45.85	40	2.5	35.15	78	586	-2.087*	0.037
ms	40	0	44.88	40	0	36.13	78	625	-2.622**	0.009
fs	40	0	42.48	40	0	38.53	78	721	-1.665	0.096

(* P<0.05 Significant, ** P<0.01 Highly Significant)

Table 3: Grades of dental caries among low birth weight and normal birth weight children

	Low Birth Weight			Normal Birth Weight			Statistical differences			
	No.	Median	Mean Rank	No.	Median	Mean Rank	df	U-Test	Z- value	P-value
d₁	40	0	45.1	40	0	35.9	78	616	-2.193*	0.028
d₂	40	2	42.61	40	1	38.39	78	715.5	-0.834	0.414
d₃	40	0	43.96	40	0	37.04	78	661.5	-1.555	0.12
d₄	40	0	41.41	40	0	39.59	78	763.5	-0.565	0.572

(* P<0.05 Significant)

Table 4: Salivary physicochemical characteristic among low birth weight and normal birth weight children

	Low Birth Weight			Normal Birth Weight			Statistical differences			
	No.	Median	Mean Rank	No.	Median	Mean Rank	df	U-Test	Z- value	P-value
Flow rate (ml/min)	40	0.307	38.88	40	0.347	42.13	78	735	-0.626	0.532
Viscosity (dyne.sec/cm²)	40	0.012	50.81	40	0.012	30.19	78	387.5	-3.970**	0.000
Cortisol (nmol/L)	40	5.52	40.99	40	5.52	40.01	78	780	-0.208	0.835

(** P<0.01 Highly Significant)

Table 5: Correlation coefficient (r) between dmfs, ds components of dental caries and salivary flow rate (ml/min), salivary viscosity (dyne.sec/cm²), and salivary cortisol (nmol/L) among low birth weight and normal birth weight children

Salivary variables	Low Birth Weight				Normal Birth Weight			
	dmfs		ds		dmfs		ds	
	r	P	r	P	r	P	r	P
Flow rate	0.014	0.934	-0.12	0.461	0.175	0.28	0.159	0.328
Viscosity	0.129	0.427	0.054	0.741	-0.11	0.501	-0.099	0.545
Cortisol	-0.279	0.081	-0.265	0.098	0.009	0.955	0.028	0.863

Table 6: Correlation coefficient (r) between salivary cortisol (nmol/L) and flow rate (ml/min) and viscosity (dyne.sec/cm²) among low birth weight and normal birth weight children

	Salivary cortisol			
	Low Birth Weight		Normal Birth Weight	
	r	P	r	P
Flow rate	-0.057	0.725	-0.178	0.273
Viscosity	0.147	0.365	0.231	0.152