

Assessment of caries experience, enamel defects and selected salivary biomarkers in children with nutritional rickets

Sara A. Jameel, B.D.S. (1)

Nadia A. AL Rawi, B.D.S, M.Sc. PH.D (2)

ABSTRACT

Background: Nutritional Rickets is a condition produced by an absence of Vitamin D, calcium or phosphate. It clues to relaxing and fading of the bones. Dental expression of children with rickets contains enamel hypoplasia and delayed tooth eruption. This study was conducted in order to assess caries experience (dmfs) and enamel defects among study and control groups, and to evaluate and compare the levels of selected salivary biomarkers between children with nutritional rickets and apparently healthy children.

Material and methods: Assessment of caries according to WHO in 1987, and assessment of enamel defects according to enamel defect index EDI of WHO in 1997. In addition a stimulated saliva samples were collected according to Palone *et al* from 30 children diagnosed with nutritional rickets and 30 control children as control group. Salivary vitamin D, calcium, phosphate and alkaline phosphatase were analyzed.

Results: Caries experience represented by dmfs was significantly higher among control group compared to study group, while enamel hypoplasia was higher in study group than control group. Salivary inorganic component (Ca, PO₄ ALP) revealed obvious variations between study and control group. Salivary vitamin D concentration was lower in study group compared with control group.

Conclusion: Based on the results, it can be concluded that nutritional rickets impact on certain salivary biomarkers which can be considered for evaluating the diagnosis and prognosis of caries experience and enamel defects in nutritional rickets children.

Keywords: Defect, biomarkers, rickets. (Received: 12/11/2017; Accepted: 1/10/2018)

INTRODUCTION

Rickets is an unstiffening of bones in children owing to shortage or diminished metabolism of vitamin D, phosphorus or calcium, possibly leading to breakages and abnormality of the bone (1,2). The prevalence of vitamin D deficiency and nutritional rickets stays to be emphasized in a number of publications, nutritional rickets is graded amongst the top five infantile diseases in developing countries (3,2). Nutritional rickets outcomes from insufficient daylight acquaintance or poor consumption of dietary vitamin D, calcium, or phosphorus (4,5,6). Nutritional Rickets typically presents at 6-24 months of age (7). Since, this is serious time period of growth of teeth, the dental manifestation embrace enamel hypoplasia, delayed formation of teeth, and increased the incidence of cavities in teeth (dental caries) (8,9).

Saliva is a compound mixture of water and organic and inorganic constituents. The capability of the public health assessment to determine the onset of the disease and treatment out comes through saliva as a non-invasive technique, the supreme important objective in the development towards the use of salivary markers can be taken in to account. (10).

In a vitamin D deficient state, intestinal Ca absorption can decrease to as low as 10-15% and there is also a decrease in total maximal reabsorption of phosphate. In this state, the low serum ionized Ca⁺⁺ level stimulates parathyroid hormone (PTH) secretion, which leads to release of Ca⁺⁺ and phosphorus (P) from bone in an attempt to maintain normal Ca⁺⁺ levels. Increased PTH levels also lead to increased urinary P excretion. Finally, the decreased levels of serum P and Ca⁺⁺ result in decreased bone mineralization (11). As far as it is known, there was no previous Iraqi studies concerning the estimation of the salivary 25(OH) vitamin D and its impact on the dental status and nutritional condition among children with nutritional rickets. Furthermore, in order to gain knowledge regarding the dental health status and certain salivary biomarkers for this target group, therefore, this study was designed and conducted.

MATERIALS AND METHODS:

Thirty children with vitamin D nutritional deficiency rickets as a study group with an age range from 1.5 to 3.5 years of both gender were examined at Paediatric Central Teaching Hospital in Nutritional Rehabilitation Care Center (N.R.C) in Baghdad, Iraq with duration of illness at least two months compared with 30 apparently healthy children they were confirmed diagnosis by special paediatrician at the same hospital as a control group. Age of children were

(1) Ministry of health, Baghdad, Iraq.

(2) Assistant Professor, Department of Pedodontics and Preventive Dentistry, College of Dentistry, University of Baghdad.

registered according to the last birthday ⁽¹²⁾. Approval was achieved from the Ministry of Health for examining the children and all the parents of children were told about the purpose of this research and agreed the research protocol.

Dental caries experience (dmfs) index were diagnosed and recorded for all children according to the criteria of WHO ⁽¹³⁾ decayed surface (ds), missing surface (ms) and filling surface (fs). All dental surfaces involved carious lesions were recorded. If there were missing teeth, they were recorded as (ms), filled surfaces as (fs) and decayed surfaces as (ds). Enamel defects were examined according to the criteria of enamel defect index of WHO ⁽¹⁴⁾. Stimulated saliva samples of children were collected according to Palone et al ⁽¹⁵⁾ at a fixed collection time (9 a.m.-12p.m.) for a chemical analysis, for this purpose the infant was seated on the mother's lap. The collected saliva was stored in a plastic test tube and freeze at -20 c after centrifugation. Inorganic phosphorus reacts with molybdic acid forming a phosphomolybdic complex. Its subsequent reduction in alkaline medium originates a blue molybdenum colour. The intensity of the colour is proportional to the inorganic phosphorus concentration in the sample. Calcium ions was determined using air – acetylene atomic absorption spectrophotometer (Buck Scientific, 210VGP, USA). ALP determined by Free phenol liberated by hydrolysis of the substrate react then with 4-aminoantipyrine in the presence of alkaline potassium ferricyanide to form red -color complex which absorbance measured at 510 nm is directly proportional to the ALP activity in the specimen, while vitamin D determined by using ELISA test. Data were expressed as mean ± SD and were analyzed statistically using SPSS (statistical package for social sciences) using parametric tests (t-test, number and percentage). The p-value was considered significant if p ≤ 0.05.

RESULTS

Table (1) revealed that caries experience represented by dmfs was significantly higher among control group compared to study group. Concerning caries experience fractions among study group the same table illustrates decay component (ds) of dmfs index represented the highest proportion and statistically significant difference, followed by missing fraction (ms) while filled fraction (fs) constituted the least proportion. For the control group (ds) component of dmfs index also represent the highest proportion followed by missing fraction (ms) and then filled fraction (fs) that constituted the least proportion. The data of the present study showed that the decayed surface value was significantly higher among the control group than the study group. The same result was found concerning missing and filling

surfaces but the difference was not significant (p>0.05).

Table (1): Caries experience of primary dentition by fractions (mean and standard deviation) between study and control group

| Variable | Study group | | Control group | | T-test | P-value |
|----------|-------------|------|---------------|------|--------|---------|
| | Mean | ±SD | Mean | ±SD | | |
| d | 1.03 | 1.22 | 2.87 | 3.81 | -2.51 | 0.017 |
| m | 0.26 | 0.58 | 0.47 | 1.01 | -0.94 | 0.352 |
| f | 0.16 | 0.37 | 0.40 | 0.85 | -1.37 | 0.180 |
| dmfs | 1.45 | 1.91 | 3.57 | 4.54 | -2.34 | 0.025 |

Regarding enamel defect among children with nutritional rickets and control children table (2) illustrates the scores of enamel defect. The current study revealed that the percentage of children with enamel defect score 0 (free from enamel defect) was more in control group than in the study group.

Concerning score 1, a higher percentage of the study group affected with enamel defect were cited under score 1 followed by score 2. In addition, children with nutritional rickets complain from score 3 were higher percentage when compared with control group.

Table (2) Distribution of children study and control group with enamel defects by scores

| Groups | Descriptive | Dental anomalies scores | | | |
|---------|-------------|-------------------------|---------|---------|---------|
| | | Score 0 | Score 1 | Score 2 | Score 3 |
| Study | N | 14 | 10 | 3 | 3 |
| | % | 46.6% | 33.3% | 10% | 10% |
| Control | N | 26 | 3 | 0 | 1 |
| | % | 86.6% | 10% | 0% | 3.3% |

The data of 60 children examined was tabulated according to the variables. In study and control group, stimulated saliva was collected and analyzed for vitamin D table (3) and inorganic calcium, phosphorous and alkaline phosphatase table (4). Concerning vitamin D the result revealed that salivary vitamin D concentration in nutritional rickets children was lower than control children with non-significant differences.

Table 3: Salivary vitamin D (mean and standard deviation) between study and control group of children

| Variable | Study group | | Control group | | T-test | P-value |
|-----------|-------------|------|---------------|------|--------|---------|
| | Mean | ±SD | Mean | ±SD | | |
| Vitamin D | 35.1 | 10.4 | 36.6 | 9.62 | 0.59 | 0.560 |

While the comparison of salivary inorganic calcium between study and control groups revealed that Ca concentration in study group was higher compared to control group but the difference was not

significant, and phosphate concentration was lower in study group than control group with highly significant differences. Concerning salivary ALP the same table revealed that the concentration in study group was higher than the control group and the difference was significance.

Table 4: Selected salivary components (mean and standard deviation) between study and control group

| Variable | Study group | | Control group | | T-test | P-value |
|-----------------|-------------|-------|---------------|-------|--------|---------|
| | Mean | +SD | Mean | +SD | | |
| Ca | 3.037 | 0.287 | 2.923 | 0.421 | 1.22 | 0.229 |
| PO ₄ | 7.521 | 0.958 | 10.30 | 2.08 | -6.66 | 0.000 |
| ALP | 2.130 | 0.315 | 1.901 | 0.408 | 2.44 | 0.018 |

DISCUSSION

The hypothesis of the current study was designated to evaluate the oral health status and investigate selected salivary biomarkers among a group of children with nutritional rickets and compare them with apparently healthy children. Caries-experience in the current study was recorded using dmfs index⁽¹³⁾. The mean dmfs for study group was lower than the mean dmfs of control group. This result may be attributed to that nutritional rickets children selected for the study were under treatment regime until they became healthy so they had calcium level higher than control group, or may be related to the fact that due to caries expansion is typically a unhurried development with numerous years of delay before a cavity is detected, consequently, the serum 25(OH)D level at the time of caries counting may or may not be illustrative of the period once caries signs established⁽¹⁶⁾.

Concerning enamel defect the result of this study showed that the percentage of teeth with score 1, score 2 and score 3 in nutritional rickets children was higher than the percentage in control group, while normal teeth percentage (score 0) appeared higher in control group than nutritional rickets children. The results related to that vitamin D deficiency causes mineralization faults in teeth, leading to poorly mineralized and hypoplastic dentin involving calcospherites rather than appropriately mineralized dentin. This mineralization blemish may disturb dental development⁽¹⁷⁾. In addition, this may be explained by a fact that enamel is somewhat thin, hypocalcified or hypoplastic, as enamel and dentin formation occur between 4 months in utero and 11 months of age, failings in primary dentition cannot be disallowed⁽¹⁸⁾.

enamel are concomitant with complications through the prenatal and primary postnatal times specially, an in utero lack in vitamin D marks in a metabolic insult to ameloblasts and accordingly, results in the development in enamel hypoplasia⁽¹⁹⁾.

The result of the current study concerning enamel defects is in agreement with Zerofsky et al and Surushi et al⁽²⁰⁻²¹⁾. In addition, other study was in agreement with the result of the current investigation, Galhotra et al that found enamel hypoplasia was noticed in 75% of children had nutritional rickets⁽²²⁾.

Vitamin D is present in saliva and the values vary throughout the day, these values obtained may relate to dietary intake of vitamin D and the subject's ethnic origin⁽²³⁾.

This results belongs to the fact that low dietary intakes associated with the increased mineralization of bone that induce reduction in 25(OH)D concentrations which together with the low calcium intakes induce rickets⁽²⁴⁾.

In addition, the findings of low vitamin D in children with nutritional rickets may be related to the short time of sun light exposure which considered as a vital factor for vitamin D intake especially for children during childhood period⁽²⁵⁾. Zerofsky et al 2015 disagreed this study when they found that no difference in salivary vitamin D concentration between children previously treated for VDD rickets and healthy children⁽²⁰⁾. Regarding concentration of salivary calcium ions, the results revealed that mean value of Ca ions concentration of study group was higher than the mean value of control group but the difference was non-significant. The findings of the current investigation may be related to the fact recorded by Thacher et al that despite 25(OH) D values being in general above 30 nmol/l, the children behaving as though they are vitamin D deficient⁽²⁶⁾. In addition, this result may be attributed to the fact that nutritional rickets children selected for the study were under treatment regime until they became healthy so they had calcium level higher control group children. This result agreed with Munns et al and Sağlam et al^(27,28), but disagreed with Schroth et al⁽²⁹⁾. In other side Hofilena showed that measurements of calcium did not demonstrate statistically significant difference in the calcium levels between the children with vitamin D deficiency and healthy children⁽¹⁷⁾. Regarding the phosphorus ions concentration in children, the mean value in nutritional rickets children was lower than control group with significant difference. The result due to the fact that phosphate concentration varies with age, with the highest concentration being in infants who require more of the mineral for bone growth and soft tissue buildup, and concentrations declining towards adulthood⁽³⁰⁾. This study agreed with Mi-Jung et al and disagreed with Sağlam et al^(31,28). Nurullah et al revealed that lower phosphorous concentration was observed in patients with vitamin D deficiency (<15 ng/mL)⁽³²⁾. Munns et al also found that phosphate concentration occurred in 7% of children with nutritional rickets he studied⁽²⁷⁾.

Regarding concentration of salivary alkaline phosphatase, the results revealed that the mean value of ALP concentration was higher among study group compared to control group with statistical significant difference. This result may be related to the fact that Alkaline Phosphatase (ALP), is an intracellular enzyme released from secondary granules of neutrophils which increase significantly with increasing inflammation. The increased activity might also be as a consequence of destructive processes in alveolar bone and metabolic changes. ALP enzyme is an indicator of higher level of cellular damage⁽³³⁾. This study was in agreement with Turan et al who was found that the highest ALP levels were detected in children with vitamin D deficiency rickets comparing with other types of rickets than healthy children⁽³⁴⁾, and agreed with Sağlam et al found that mean value of alkaline phosphatase in rickets children was higher than mean value of control group and the difference was significant⁽²⁸⁾. Nurullah et al revealed that higher level of ALP was observed in children with vitamin D deficiency (<15 ng/mL)⁽³²⁾. Munns et al also found in their study that ALP concentration was high in 100% of children with nutritional rickets⁽³⁵⁾. However, Zerofsky et al concluded that there were no significant difference in bone specific alkaline phosphatase among children with nutritional rickets⁽²⁰⁾. Galhotra et al found an abnormal alkaline phosphatase level in children with vitamin D deficiency rickets⁽²²⁾.

REFERENCES

1. Choudhury S, Jebasingh KF, Ranabir S, Singh TP. Familial vitamin D resistant rickets, End-organ resistance to 1,25-dihydroxyvitamin D, Indian J Endocrinol Metab, 2013; 17:S224-7.
2. Winzenberg T, Jones G. Vitamin D and bone health in childhood and adolescence, Calcif Tissue Int, 2013;92:140-50.
3. Thacher TD, Fischer PR, Pettifor JM. Vitamin D treatment in calcium-deficiency rickets, A randomized controlled trial, Arch Dis Child, 2014; 99:807-11.
4. DeLucia MC, Mitnick ME, Carpenter TO. Nutritional rickets with normal circulating 25-hydroxyvitamin D: a call for reexamining the role of dietary calcium intake in North American infants, J Clin Endocrinol Metab 2003; 88:3539-45.
5. Shah M, Salhab N, Patterson D, Seikaly MG. Nutritional rickets still afflict children in north Texas, Tex Med 2000; 96:64-8.
6. Carvalho NF, Kenney RD, Carrington PH, Hall DE. Severe nutritional deficiencies in toddlers resulting from health food milk alternatives, Pediatrics 2001; 107:E46.
7. Prentice A. Nutritional rickets around the world, J Steroid Biochem Mol Biol, 2013; 136:201-6.
8. Davit-Béal T, Gabay J, Antonioli P, Masle-Farquhar J, Wolikow M. Dental complications of rickets in early childhood: Case report on 2 young girls, Pediatrics 2014; 133:e1077-81.
9. Zambrano M, Nikitakis NG, Sanchez-Quevedo MC, Sauk JJ, Sedano H, Rivera H. Oral and dental manifestations of vitamin D-dependent rickets type I: Report of a pediatric case, Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003; 95:705-9.
10. Sun, F., & Reichenberger, E. J. Saliva as a source of genomic DNA for genetic studies, Review of current methods and applications, Oral Health Dent Manag, 2014; 13(2), 217-222.
11. Misra M, Pacaud D, Petryk A, Solberg FC, Kappy M. Vitamin D deficiency in children and its management: Review of current knowledge and recommendations. Pediatrics 2008; 122:398-417.
12. World Health Organization, Oral Health Surveys, Basic Methods, 4th edition, Geneva 1997.
13. World Health Organization. Oral health surveys, Basic methods, 3rd edi. 1987.
14. World Health Organization, Oral Health Surveys, Basic Methods, 3th edition, Geneva 1997.
15. Palone MRT, Vargas VPS, Silva TR. Development of experimental model for assessing levels of microorganisms in saliva of children with cleft palate, Rev Fac Cien Med Univ Nac Cordoba, 2016; 73:219-220.
16. Schroth R, Levi J, Sellers E, Friel J, Kliewer E, Moffatt M. Vitamin D status of children with severe early childhood caries, A case control study, Biomed Central Pediatrics, 2016; 13:1-8.
17. Vanessa O. Hofilena. A Comparison of Vitamin D Levels in Children with Early Childhood Caries, Virginia Commonwealth University, 2015.
18. AL-Jundi SH, Hazza'a AM. Dental Development in Patients with Hypophosphatemic Rickets, IJCPD 2013; 3(1):1-4.
19. Schroth R, Levi J, Sellers E, Friel J, Kliewer E, Moffatt M. Vitamin D status of children with severe early childhood caries, A case control study, Biomed Central Pediatrics, 2015; 13:1-8.
20. Zerofsky M, Mark Ryder, Charles B. Stephensen, Janet King, and Ellen B. Fung. Effects of early vitamin D deficiency nutritional rickets on bone and dental health, growth and immunity Genicol T, Cooper-White J, Beyerlein PK, Kostner K, Punyadeera C. Diagnostic potential of saliva. Current state and future applications. Clinical Chemistry 2013; 57(5): 675-687.
21. Surushi B, Charles B. Stephensen, Janet King, and Ellen B. Fung Effects of early vitamin D deficiency rickets on bone and dental health, growth and immunity 2015.
22. Virat Galhotra1, Pooja Ahluwalia1, Sunaina Jodhka, Sumit Sethi, Singh Gambhir. Nutritional rickets and dental development, Departments of Pedodontics and Preventive Dentistry and Public Health Dentistry, Bhojia Dental College and Hospital, Baddi, Himachal Pradesh, India 2012.
23. Lavie CJ, Lee JH, Milani RV. Vitamin D and Cardiovascular Disease, Journal of the American College of Cardiology, 2011; 58:1547-56.
24. Agela Faierny and P. W. Saphier. Studies on the measurement of 25-hydroxy vitamin D in human

- saliva, Department of Chemical Pathology, St. Mary's Hospital Medical School, London W2 1PG 1986.
25. Pettifor JM. Rickets and vitamin D deficiency in children and adolescents, *Endocrinol Metab Clin North Am* 2005; 34:537-53.
 26. Thacher TD, Fischer PR, Pettifor JM. Vitamin D treatment in calcium-deficiency rickets: a randomised controlled trial, *Arch DisChild*. 2010; 50:206-13.
 27. Munns C, Zacharin MR, Rodda CP, et al. Prevention and treatment of infant and childhood vitamin D deficiency in Australia and New Zealand, a consensus statement, *Med J Aust* 2007; 185: 268-272.
 28. Saglam Y, Kizildag H, Toprak G, Alp NB, Yalcinkaya EY. Prevalence of vitamin D insufficiency in children with forearm fractures, *J Child Orthop* 2017;11: 180-184.
 29. Oginni LM, Sharp CA, Badru OS, Risteli J, Davie MWJ, Worsfold M. Radiological and biochemical resolution of nutritional rickets with calcium, *Arch Dis Child* 2003; 88:812-17.
 30. Alizadeh Naderi AS, Reilly RF. Hereditary disorders of renal phosphate wasting, *Nat Rev Nephrol*, 2010; 6:657-665.
 31. Mi jung, Pencina MJ, Booth SL, et al. Vitamin D deficiency and risk of cardiovascular disease circulation, 2008;117:503-511.
 32. Nurullah Celik, Nesibe Andiran, Halise Akca, Guzide Doğan Fatih . Vitamin D Deficiency in Children and Adolescents. University Faculty of Medicine, Department of Pediatric Endocrinology, Ankara, Turkey, Fatih University Faculty of Medicine, Department of Pediatrics, Ankara, Turkey 2012.
 33. Priti P, Basguda P. Saliva: A diagnostic biomarker of periodontal diseases, *J Indian Soc Periodontol*, 2011;15 (4):310-17.
 34. Turan S, Topcu B, Gökcüoğlu I, Güran T, Atay Z, Omar A. Serum alkaline phosphatase levels in healthy children and evaluation of alkaline phosphatase z-scores in different types of rickets, *J Clin Res Pediatr Endocrinol* 2011; 3(1):7-11.
 35. Munns CF, Simm PJ, Rodda CP, et al. Incidence of vitamin D deficiency rickets among Australian children, an Australian Paediatric Surveillance Unit study, *Med J Aust*, 2012; 196:466-8.

الخلاصة

خلفية الموضوع: الكساح الغذائي هو حالة تنتج عن غياب فيتامين د أو الكالسيوم أو الفوسفات. إنه دليل على تلاشي العظام. يحتوي تعبير الأسنان للأطفال المصابين بالكساح على نقص تنسج المينا وبزوغ الأسنان المؤجل. في التشخيص عن طريق الفم، هناك تجربة غير محدودة لتحديد المرقمات الحيوية لفحص وتقييم عمل المرض. ومع ذلك، يمكن أن تساعد المؤشرات الحيوية للعبية أيضاً كوسيلة قيمة لتقدير كفاءة العلاج.

الهدف من الدراسة: لتقييم مؤشر التسوس (dmfs) وعبوب المينا بين مجموعات الدراسة والمجموعة الضابطة وكذلك لتقييم ومقارنة مستويات العلامات الحيوية للعبية المختارة بين الأطفال الذين يعانون من الكساح الغذائي والأطفال الأصحاء.

المواد والطرق: تم جمع عينات من اللعاب المحفز وفقاً لـ Palone et al 2016 من 30 طفلاً تم تشخيصهم بالكساح الغذائي و 30 طفلاً أصحاء كمجموعة ضابطة. تم تحليل فيتامين (د) اللعاب، الكالسيوم والفوسفات و الفوسفات القلبية. وبالإضافة إلى ذلك، تقييم النخر وفقاً لمنظمة الصحة العالمية (1987)، وتقييم عبوب المينا وفقاً لمؤشر عيب المينا EDI (منظمة الصحة العالمية 1997).

النتائج: كانت تجربة التسوس التي تمثلها dmfs أعلى بشكل ملحوظ بين مجموعة الاصحاء مقارنة بمجموعة الدراسة، بينما كان نقص تنسج المينا أعلى في مجموعة الدراسة مقارنة بمجموعة الاصحاء. أظهر اللعاب في المكون العضوي (Ca، PO4 ALP) اختلافات واضحة بين مجموعة الدراسات ومجموعة الاصحاء. وكان تركيز فيتامين D اللعابي أقل في مجموعة الدراسة مقارنة مع مجموعة الاصحاء.

الاستنتاجات: استناداً إلى النتائج، يمكن الاستنتاج أن الكساح التغذوي يؤثر على بعض المرقمات الحيوية للعبية التي يمكن اعتبارها لتقييم التشخيص والتنبؤ بتجربة Careis وعبوب المينا في الكساح التغذوي للأطفال والأطفال الأصحاء.