RESEARCH ARTICLE

EVALUATION AND ASSOCIATION OF IRON DEFICIENCY ANAEMIA WITH SALIVARY pH AND BUFFERING CAPACITY

Background: Dental caries is a microbial disease characterized by demineralization of the inorganic portion and destruction of the organic substance of the tooth. Dental caries, its resulting discomfort and pain can interfere with their nutritional intake including iron which results in iron deficiency anaemia.
Aims & Objective: To evaluate the association between iron deficiency anaemia with salivary pH and buffering capacity before and after iron supplementation in children of 6-12 years of age group.
Materials and Methods: Dental caries was recorded as per World Health Organization criteria. Blood was drawn from the patients for the estimation of Hb, MCV, MCH and serum ferritin levels to diagnose the iron deficiency anaemia. Unstimulated Saliva was assessed for, salivary pH and buffering capacity. Blood as well as salivary parameters were measured in two occasions, before beginning of the treatment for iron deficiency anaemia and after completion of treatment of iron deficiency anaemia. The obtained data was tabulated and statistical analysis was done.
Results: On statistical evaluation by students paired 't' test, the parameter like Hb, MCV, MCH, Serum ferritin, salivary pH, and buffering capacity after the iron therapy showed statistical significance, ($p<0.001$). It was also found that the correlation and association of iron deficiency anaemia was evaluated with the Hb, MCV, and salivary pH.
Conclusion: Iron deficiency anaemia, the most commonly recognized form of nutritional deficiency, is prevalent among infants and young children as hypochromic microcytic anaemia. Due to change in the salivary pH and buffering capacity the risk of developing dental caries as well as deterioration of oral health is evident. Hence evaluation of these factors in saliva that may increase the risk of individuals to dental caries, can pave way to make recommendations that will cater specifically to needs of an individual. Iron therapy in iron deficiency anaemia can increase the levels of Hb, MCV, MCH, Serum ferritin, salivary pH and buffering capacity. Long term effect of iron deficiency anaemia on oral health is still needed to be explored.
Key Words: Unstimulated Whole Saliva; Salivary pH; Buffering Capacity; Caries; Iron Deficiency; Serum Ferritin

Taranath Mahantesha[1], KM Parveen Reddy[1], Vijaya Prasad Kamavaram Ellore[1], Naveen Kumar Ramagoni[1], Vijaynath Iitagi[2], Anitha KS[2]

[1] Navodaya Dental College and Hospital, Raichur, Karnataka, India

[2] Navodaya Medical College Hospital and Research Center, Raichur, Karnataka, India

Correspondence to: KM Parveen Reddy (drpavanireddy@yahoo.com)

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INTRODUCTION

Oral cavity is distinctive ecosystem harvesting different types of hard and soft tissues and harbors plethora of microorganisms and is specialized to perform wide variety of functions.[1] Among the oral diseases, dental caries is the most common chronic disease of mankind.[2] Studies have shown that saliva serves as a mirror of the body's health as it contains proteins, hormones, antibodies and other molecules that are frequently measured in standard blood tests to monitor health and disease. However, unlike whole blood, saliva is easy to collect, less painful to the patient and is less infectious for the health care provider. Healthy individuals produce about a liter and a quarter of saliva per day. Nearly all analytes that are in blood are also present in saliva. Recently it has been claimed that the imbalances in levels of free radicals, reactive oxygen species and antioxidants in saliva may play an important role in the onset and development of dental Caries. Hence evaluation of those factors in saliva that may increase the risk of individuals to dental caries, can pave way to make recommendations that will cater specifically to needs of an individual.[3]

In one of the study it was observed that salivary flow rate, pH, buffering capacity and calcium are slightly decreased in caries active children as compared to caries free children however, the difference was not found to be statistically significant.[1] In general, higher the flow rate, faster the clearance and higher the buffering capacity and thus lesser microbial attacks.

Dental caries is an irreversible microbial disease of the calcified tissues of teeth characterized by demineralization of the inorganic portion and destruction of the organic substance of the tooth which often leads to cavitation.[4] Dental caries, its resulting discomfort and pain can interfere with their nutritional intake including iron which results in iron deficiency anaemia.[5]

It affects persons of both sexes in all races, all socio-economic strata and every age group. As children reach school age, they will have an increasing incidence of carious lesions because of change in dietary habits which

includes refined carbohydrates and sweeteners.[4] It is also profoundly affected by other factors like oral hygiene and saliva. The saliva circulating in the mouth at any given time is termed as whole saliva and comprises a mixture of secretions from the major, minor salivary glands and traces from the gingival crevicular fluid. To a large extent it promotes oral health, whereas lack of its secretion contributes to the disease process.[4,6] Saliva is a heterogeneous fluid comprising of proteins, glycoproteins, electrolytes, small organic molecules and compounds transported from blood.[7]

No other etiological factor can influence the outcome of dental caries, as much as the saliva can do. The epidemiological surveys and experimental studies are gradually increasing our understanding and awareness of the impact of saliva on oral health.[4] Salivary hypo function is associated with oral and pharyngeal disorders which require early diagnosis and intervention for better patient care.[8] Salivary flow rate is reduced due to various factors such as insufficient food intake and nutrition. One such nutrition related factor is iron deficiency anaemia.[9]

Iron deficiency is a state in which there is insufficient iron to maintain normal physiologic functions. Iron deficiency results from inadequate iron absorption to accommodate an increase in requirements attributable to growth or resulting from a long-term negative iron balance. Iron deficiency affects mental development[10], visual, auditory functions and weakly associated with poor cognitive development in children[11].

Children with ID (iron deficiency) tend to fatigue easily and have a decreased appetite which is associated with irreversible developmental and cognitive delays.[12] Many studies have been carried out in relation to physicochemical properties of saliva (like, buffering capacity, pH, and concentration of components of saliva) and their relation with dental caries in adults and few in children.[13] Very few studies have been done to correlate salivary function and IDA (iron deficiency anaemia). So present study was undertaken to evaluate and correlate IDA with Salivary pH and buffering capacity, as these parameters are definitely affected in iron deficiency anaemia.[14]

MATERIALS AND METHODS

This in vitro study was conducted on 150 children of age group between 6 and 12 years reported to the Department of Pedodontics and Preventive Dentistry, Navodaya Dental College and Hospital. The study protocol was approved by the Ethics Committee of Navodaya dental College, Raichur. Informed consent was obtained from parent/guardian of each child.

The blood parameter analysis and salivary analysis was carried out. An elaborate questionnaire was prepared to collect the information regarding personal, oral and general health. Blood and salivary parameters were measured in two occasions that is before beginning of the treatment for iron deficiency anaemia and after completion of treatment of iron deficiency anaemia.

To confirm the iron deficiency anaemia, Iron deficiency was considered to be present when, hemoglobin was less than 10 g/dl, MCV level less than 80 fL, and MCH level less than 30 pg.[15] To assess iron deficiency, serum ferritin level estimation was done. Cubital venous blood was drawn into 5 ml vacutainer tubes without anticoagulants.[16] Coagulated blood was centrifuged at 3000 rpm for 5 minutes and clear plasma obtained was appropriately labeled, which was analyzed by fully automated biochemistry analyzer.

For salivary parameters the unstimulated saliva was collected and pH and buffering capacity were assessed. Electronic pH meter was used to estimate pH, and Ericson method for estimating buffer capacity.[14] Parameters like salivary pH, buffer capacity and Serum ferritin levels were estimated to assess their role as markers of iron deficiency anaemia and dental caries.[14] The collected saliva was mixed by inverting the tube twice. Ericson method: 1.0 ml of the saliva was transferred to 3.0 ml HCl (0.0033mol per L for unstimulated saliva). For preventing the foaming, one drop of 2-octanol is added and mixed for 20 minutes to remove CO2. Final pH in the saliva was evaluated electrometrically.[14] Typically, for iron therapy up to 300 mg of elemental iron per day was given in case of iron deficiency anaemia.[17]

The data thus obtained were subjected to statistical analysis by using Student's paired 't' test. Pearson's correlation coefficient (r) was done to find out relationship between iron deficiency anaemia with other parameters. A p-value less than 0.05 were considered as significant. Similarly after three months, the saliva and blood sample were collected from the study group for assessing the evaluation and correlation between Iron Deficiency anaemia, salivary pH and buffering capacity. The data thus obtained were subjected to statistical analysis by using Students paired 't' test and Pearson

correction was applied. The results were tabulated using SPSS verse 16 software.

RESULTS

Data obtained after the medication for the period of three months are given in Table 1. On observation it was found that Mean levels of Hb, MCV and pH after treatment of IDA, were increased significantly and showed positive relationship. Whereas mean levels of MCH, Serum Ferritin, Buffering capacity of saliva after treatment were increased significantly, but did not show relationship with iron deficiency anaemia when compared to baseline which were statistically significant (p<0.001) (Table 1 and 2).

Table-1: Comparison of iron deficiency anaemia and salivary parameter in children of 6 to 12 year age before and after treatment (Mean ± SD)

Laboratory Values	Before Treatment	After Treatment	95% CI	p-value
HB	7.6 ± 1.15	9.03 ± 1.04	1.26-1.51	<0.0001
MCV	69.94 ± 7.8	86.04 ± 4.66	14.88-17.32	<0.0001
MCH	23.68 ± 1.68	28.04 ± 1.18	4.06-4.65	<0.0001
Serum ferritin	7.57 ± 1.34	21.99 ± 6.92	13.41-15.43	<0.0001
pH	7.06 ± 0.4	7.22 ± 0.3	0.09-0.22	<0.0001
Buffering capacity	5.08 ± 0.5	5.2 ± 0.4	0.07-0.17	<0.0001

Paired 't' test; P <0.05 significant; P>0.05 not significant

Table-2: Relationship between iron deficiency anaemia with other parameters by Pearson's correlation coefficient (r)

Parameters	r	p-value	Remarks
HB>10	0.755	0.0001	HS
MCV<80	0.083	0.31	NS
MCV>80	0.18	0.03	Significant
MCH<30	-0.076	0.36	NS
MCH(20-30)	0.095	0.25	NS
serum ferritin (<10 ng/ml)	-0.02	0.808	NS
serum ferritin (>10 ng/ml)	-0.064	0.44	NS
pH before	0.149	0.07	NS
pH after	0.195	0.02	Significant
Buffering capacity Before	0.014	0.86	NS
Buffering capacity After	0.029	0.72	NS

From Pearson's correlation coefficient, it is found the ID significantly had positive relationship with Hb>10mg/% (p<0.0001), MCV>80 (p=0.03), and pH after (p=0.02).

Table-3: Buffer for unstimulated saliva

Final pH value	Evaluation
more than 4.75	High
4.25 - 4.75	Normal
3.50 - 4.24	Low
less than 3.50	very low

Pearson's correction was used in this study which is an adjustment made to P values. As shown in table 1 and 2 with regards to Hb, the mean ± S.D. of Hb % in cases, mean Hb of iron deficiency anaemia before treatment was 7.6 ± 1.15, after treatment 9.03 ± 1.04 with 95% CI for the difference (1.26-1.51). The mean ± S.D. of MCV % in the cases, before treatment was 69.74 ± 7.8, after treatment 86.04 ± 4.66 with 95% CI for the difference (14.88 – 17.32). MCH, the mean ± S.D. of MCH % in cases, before treatment was 23.68 ± 1.68, after treatment 28.04 ± 1.18 with 95% CI for the difference (4.06 – 4.65). Serum Ferritin, the mean ± S.D. of Serum Ferritin % before treatment was 7.57 ± 1.34, after treatment 21.99 ± 6.92 with 95% CI for the difference (13.41 – 15.43). The mean ± S.D. of pH before treatment was 7.06 ± 0.4, after treatment 7.22 ± 0.3 with 95% CI for the difference (0.09 to 0.22). The mean ± S.D. of Buffering Capacity of Saliva % in cases, before treatment was 5.08 ± 0. , after treatment 5.2 ± 0.4 with 95% CI for the difference (0.07 – 0.17).

As shown in table 2 , relationship between iron deficiency anaemia with other parameters from Pearson's correlation coefficient, it is found that ID iron deficiency significantly had positive relationship with Hb>10mg/% (p<0.0001), MCV>80 (p=<0.0001), and pH after treatment (p<0.0001)

DISCUSSION

Dental caries is the most common chronic disease of mankind.[14] Dental caries is multifactorial, transmissible, and diet bacterial disease , and diet plays a critical role in the development and clinical features of this infection.[18] Saliva is the important factor which influences the development of dental caries as teeth are in constant contact and are bathed by the saliva.

Parameters like salivary pH & buffer capacity were estimated to assess their role as markers of iron deficiency anaemia and dental caries.[14] So present study has been done to make an attempt to evaluate association between iron deficiency anaemia with salivary pH, and buffering capacity in children of 6 to 12 year age.

Anaemia prevalence in young children continues to remains over 70% in most parts of India and Asia despite a policy being in place and a program that has been initiated for a long time.[19]

Parameters like, salivary pH & buffer capacity and Serum ferritin levels are the markers of iron deficiency anaemia and dental caries.[14] Salivary buffering capacity has been identified as one of the many factors that may affect an individual's caries risk.[20] The ability of saliva to buffer acids is essential for maintaining pH values in the oral environment above the critical pH, thereby protecting teeth against demineralization. Saliva has a buffer capacity which neutralizes acids in the mouth. This capacity is based on several systems such as the

phosphate system and the carbonic acid/bicarbonate system. It is imperative that measurement of salivary buffering capacity is accurate so that appropriate preventive management may be implemented for individual patients. Another Study done by Zlotkin et al in 2003[21] stated that malnutrition, such as iron deficiency, often impairs salivary gland function causing reduced salivary secretion and low buffering capacity. Oral cavity is quite frequently exposed to components whose pH differs from normal pH (6.5 – 7.5) of saliva and these components may cause damage to teeth or mucosal surface.

Buffering agents in saliva, however try to bring the pH back to the normal range as fast as possible. In resting saliva the major buffering agent is inorganic phosphate and in stimulated saliva it is carbonic acid / bicarbonate system. At very low pH (4-4.5) salivary proteins also display some buffering action.[22] Studies have shown that patient with low or no caries activity had a resting salivary pH of around 7.0. Those with extreme caries activity had a resting pH below critical pH 5.5.[4]

In our study it is observed that the level of Hb, MCV,MCH, serum ferritin and salivary pH buffering capacity after the treatment of iron deficiency anaemia were improved and showed statistical significant ($P<0.0001$). The Pearson's correlation coefficient table shows that the ID(iron deficiency) significantly had positive relationship with Hb>10mg/% ($p<0.0001$), MCV>80 ($p=0.03$), and pH after treatment ($p=0.02$) [Table 2].

CONCLUSION

Iron deficiency anaemia leads to weakness, poor physical growth, and a compromised immune system with decreasing ability to fight infections and increasing morbidity. Iron deficiency anaemia, the most commonly recognized form of nutritional deficiency, is prevalent among infants and young children as hypochromic microcytic anaemia. . Due to change in the salivary ph and buffering capacity, the risk of developing dental caries as well deterioration of oral health is evident. Hence evaluation of these factors in saliva that may increase the risk of individuals to dental caries, can pave way to make recommendations that will cater specifically to needs of an individual. Further long term study has to be conducted to explore the effect on iron deficiency anaemia.

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