EVALUATION OF THE ASSOCIATION BETWEEN ANAEMIA AND DENTAL CARIES IN ADULT PATIENTS
ABSTRACT

Background:

Dental caries and anaemia are two major global health issues. Anaemia as a potential risk factor for dental caries remains unexplored especially in an adult population. The purpose of the present study was to investigate a possible association between dental caries and anaemia in adult patients.

Methods:

This study screened 403 adults who had reported for routine blood investigation with regard to their haemoglobin (Hb) and dental caries status. The caries status was recorded for the individual participants based on their DMFT index and anaemia was recorded based on the haemoglobin levels, i.e., male below 13 g/dl and female below 12 g/dl, respectively, as according to the WHO criteria. Mean corpuscular volume (MCV) values were recorded to classify the type of anaemia. Based on the screened reports, the participants were segregated into two groups; those with anaemia (Anaemic group) and those without anaemia (Non-anaemic group) according to ages in decade. The data was statistically analyzed using SPSS (IBM SPSS Statistics for Windows, Version 23.0, Armonk, NY: IBM Corp, Released 2015).

Results:

The mean DMFT index was 5.2 for both the genders in the anaemic group, which was 5.2 in females and 4.0 in males. In non-anaemic group, the mean DMFT index was 3.48 both the genders, which was 3.8 in females and 3.3 in males. The mean DMFT score
was 5.20±4.97 for anaemic group and 3.48±3.10 for non-anaemic group. Mann-Whitney U test showed a statistically significant association between dental caries and anaemia (p<0.001). The p-value was calculated for each age strata so as to eliminate the confounding effect of age on dental caries and anaemia and was found to be statistically significant.

**Conclusion:**

The study results confirmed a strong association between dental caries and anaemia in adults.
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INTRODUCTION

The Federation Dentaire Internationale (FDI) defined oral health as “multifaceted and includes the ability to speak, smile, smell, taste, touch, chew, swallow, and convey a range of emotions through facial expressions with confidence and without pain, discomfort, and disease of the craniofacial complex.”¹

The World health organisation (WHO) states that among the various threats to oral health, dental caries remains the most prevalent disease globally ² and is defined as a “localised destruction of susceptible dental hard tissues by acidic by-products from bacterial fermentation of dietary carbohydrates.”³ The prevalence of dental caries in India has been estimated to be around 27 to 64% in children and 26 to 83% in adults.⁴

Despite being the most common preventable disease,³ about half of the global population is still affected by dental caries.² This is due to the lack of complete understanding of its etiology. Dental caries is considered as a multifactorial disease involving known biological factors, genetics and social modifiers. The well known risk factors of dental caries include cariogenic microflora, increased intake of refined sugars, poor oral hygiene, inadequate salivary flow, poor brushing habits, low socio-economic status and a low literacy level.³ Apart from these traditional risk factors of dental caries, anaemia remains underestimated as a potential risk factor for dental caries though recent studies have suggested a significant association between severe early childhood caries and anaemia in children.⁵-⁷

However, the association of dental caries with anaemia in adult patients has not been studied unlike the childhood form of the disease, though both dental caries and anaemia are known to affect the well-being of adults.
Anaemia is a condition in which afflicted individuals have too few RBCs or haemoglobin functioning at a suboptimal level. Among the various types of anaemia, iron deficiency anaemia remains the most common, afflicting about one third of the population worldwide. The global prevalence of anaemia has been estimated as 24.8% as per the 1993-2005 WHO database on anaemia. The 1993-2005 WHO survey has categorised the countries on the basis of prevalence of anaemia. In India, the prevalence of anaemia is more than 40% which is perceived as a severe public health problem.

Thus, despite the epidemic nature of dental caries and anaemia, the association between the two conditions in adult population remains obscure. This oral-systemic relationship deserves to be explored for formulating an etiological hypothesis and for planning prevention strategies in future.

Therefore, purpose of the present study is to analyse the relationship between decayed, missing, filled tooth (DMFT) scores and haemoglobin levels in adult patients in order to determine the association of dental caries and anaemia in adults.
AIM AND OBJECTIVE

AIM

Aim of the present study is to analyse the association between dental caries and anaemia in adult patients attending the dental out-patient clinics at Tamil Nadu Government Dental College and Hospital, Chennai.

OBJECTIVE

The objective of the study is to determine the relationship between the decay, missing, filled tooth (DMFT) scores, haemoglobin (Hb) levels and mean corpuscular volume (MCV) in adult patients.
REVIEW OF LITERATURE

DENTAL CARIES

Dental caries is a chronic microbial disease of the dental hard tissues. Various theories have been proposed to explain its etio-pathogenesis with none being universally accepted, though the chemo-parasitic theory by W.D. Miller became a major scaffold for the present-day understanding of cariogenesis.\textsuperscript{10}

The complicated etiology of dental caries includes an inter-play of many factors, among which the three major factors are cariogenic microflora (streptococcus mutans, actinomyces and lactobacillus), suitable environment (dietary carbohydrates, plaque quantity and quality, bacterial enzymes) and a susceptible host (tooth mineral composition, tooth ultrastructure, saliva and host immunity) over a prolonged period of time. The other minor contributing factors include social class, income, knowledge, behaviour and education (Figure-1).\textsuperscript{10}

The disease process is initiated by the cariogenic micro-organisms that reside in a complex bio-matrix called the dental plaque, which provides a favourable micro-environment for the micro-organisms to survive and exhibit their virulence. These micro-organisms act on a suitable substrate (carbohydrates) to produce weak organic acids such as lactic, acetic, formic, and propionic acids,\textsuperscript{11} thereby decreasing the local pH which disturbs the mineral homeostasis between the tooth minerals and the oral fluids.

Demineralisation occurs when the pH drops below the critical pH which might range from 5.5 to 6.5 depending on the concentration of calcium and phosphate ions in the oral fluids.\textsuperscript{12} This demineralisation is not a continuous process but alternates with periods of remineralisation, which occurs when the local pH is restored to normal level
by the salivary buffers. The salivary buffers are prevented from reaching the acids by the plaque matrix, thereby allowing the demineralisation to outlast. Thus caries is a dynamic process with a resultant mineral loss from the tooth structure and eventual cavitation, which can be reversed at earlier stages if the mineral homeostasis is restored back.

Demineralisation in dental caries can be appreciated only at the ultra-structural level in the earlier stages (Figure-2). With continuous demineralisation, dental caries is evidenced clinically as white-spot lesion which leads to frank cavitation if therapeutic interventions are not made to revert the disease process.
Figure-1 Major and minor contributing factors for dental caries.

Major factors are placed in the inner circle and minor factors are placed in the outer circle.

Courtesy: Usha et al., 2009\textsuperscript{10}
Figure-2 Demineralisation at the ultra-structural level.

Courtesy: Featherstone et al., 2008\textsuperscript{11}
RAMPANT CARIES

Rampant caries is an acute and rapidly progressing variant of the disease affecting almost all the erupted teeth, often involving surfaces that are normally resistant to decay. It is characterised by coronal tissue destruction and an early pulpal involvement.\textsuperscript{14} It is commonly observed in deciduous dentition in the paediatric population and has been termed as severe early childhood caries, whereas the adult form of rampant caries in permanent dentition is not so common.\textsuperscript{15}

Severe early childhood caries (S-ECC) is defined as “any sign of smooth-surface caries in a child younger than three years of age, and from ages three through five, one or more cavitated, missing (due to caries), or filled smooth surfaces in primary maxillary anterior teeth or a decayed, missing, or filled score of greater than or equal to four (age 3), greater than or equal to five (age 4), or greater than or equal to six (age 5)”. It is most often associated with frequent night time bottle feeding with sweetened liquids and snacking.\textsuperscript{16}

Adult rampant caries is defined as “multiple open coronal carious cavities (at least 8), including caries of an anterior tooth (mandibular or maxillary incisor, or canine) in patients aged 16 years or older”. It is seen often associated with salivary gland hypofunction.\textsuperscript{15}
Association between anaemia and dental caries in adults

ANAEMIA

Anaemia is a condition in which the number of red blood cells (RBCs) or haemoglobin concentration is insufficient, consequently impairing the capacity of the blood to transport oxygen around the body to meet physiologic needs, which vary by age, sex, altitude, smoking, and pregnancy status.\textsuperscript{17,18} It is diagnosed when the haemoglobin concentration falls below the normal levels. The World Health Organization (WHO) defines anaemia as a haemoglobin concentration less than 13 g/dL in adult males and less than 12 g/dL in non-pregnant adult females.\textsuperscript{19}

The reduced oxygen supply to the tissues, results in the clinical signs and symptoms of anaemia.\textsuperscript{20} Most patients with anaemia are asymptomatic till the haemoglobin concentration falls below 7g/dL due to the compensatory mechanisms. The commonly encountered symptoms of anaemia are dyspnoea, dizziness, fatigue, increased thirst and impaired cognition.\textsuperscript{19} A complete history and clinical examination with laboratory investigation is necessary to determine the cause and type of anaemia.

A past medical history for blood loss in the form of heavy menstrual bleeding, haematuria, malena, haematochezia, haemoptysis, haematemesis, and haemorrhage following trauma or recent surgery should be elaborated. Family history is also necessary to rule out inherited forms of anaemia like sickle cell disease.\textsuperscript{19}

Pallor in mucosae, scleral icterus, koilonychia, tachycardia are the most common signs elicited during the clinical examination of anaemic patients. Severe forms of anaemia can present with cardiac murmurs, pulmonary crackles and organomegaly.\textsuperscript{19}

Reduced erythropoiesis, haemolysis, and blood loss are the three major mechanisms underlying the development of anaemia and are brought about by nutritional deficiencies, genetic haemoglobinopathies, acute and chronic infections and other
diseases. Among the various causes, iron deficiency accounts for more than half the cases of anaemia.  

Anaemia can be classified in several ways. Based on the clinical presentation and investigations, anaemia can be classified as acute anaemia or chronic anaemia.

**Acute anaemia**

The acute loss of RBCs is the major mechanism underlying acute anaemia which can be due to several reasons like haemorrhage following a trauma, internal bleeding like gastrointestinal bleeding, ruptured aneurysm, genitourinary bleeding. Other rare causes include acute splenic sequestration in sickle cell disease and autoimmune haemolytic anaemias.

**Chronic anaemia**

Anaemia caused by impaired production of RBCs or destruction of RBCs is most often the mechanism underlying chronic forms of anaemia. Nutritional deficiencies such as that of iron, vitamin B12, and folate result in impaired erythropoiesis which is the most common cause of chronic anaemia. Abnormal haemoglobin synthesis as in haemoglobinopathies like thalassaemia leads to decreased production of RBCs resulting in chronic anaemia. The destruction of RBCs as in hemolysis of sickled RBCs in sickle cell disease also results in chronic anaemia.

The size of the RBCs as determined by the mean corpuscular volume (MCV) or a peripheral blood smear classifies anaemia as microcytic, normocytic and macrocytic anaemia, which is the first clue to diagnose the underlying etiology of anaemia. Therefore, initial evaluation of anaemia based on the mean corpuscular volume (MCV)
into normocytic, microcytic and macrocytic anaemia is an accepted practice in routine laboratory investigations.\textsuperscript{19}

**Microcytic anaemia**

Anaemia in which the RBCs synthesized are smaller in size as determined by a reduced MCV (lesser than 80 fL) is called microcytic anaemia.\textsuperscript{21} The most common microcytic anaemia is the iron deficiency anaemia with others being thalassaemia, sideroblastic anaemia, anaemia due to lead poisoning or chronic diseases.\textsuperscript{8,22}

**Normocytic anaemia**

Anaemia in which the RBCs synthesized are normal in size as indicated by an MCV between 80 to 100 fL is called normocytic anaemia. Haemolytic anaemia as in sickle cell disease, spherocytosis, anaemia of chronic diseases, renal anaemia, acute anaemia following blood loss are some examples of normocytic anaemia. Anaemia of chronic disease can be either microcytic or normocytic in nature.\textsuperscript{19,22}

**Macrocytic anaemia**

Anaemia in which the RBCs synthesized are larger in size as determined by an increased MCV (greater than 100fL) is termed as macrocytic anaemia. Anaemia due to vitamin B-12 deficiency or folate deficiency, liver diseases and aplastic anaemia are some examples of macrocytic anaemia.\textsuperscript{19,22}

Irrespective of the underlying cause of anaemia, haemoglobin concentrations are considered the most reliable indicator of anaemia,\textsuperscript{9} though additional investigations are required to rule out its etiology. The normal haemoglobin concentrations as established by the WHO\textsuperscript{23} and centres for disease control (CDC)\textsuperscript{24} are given in table 1.1 and 1.2.
Table 1.1 Normal haemoglobin concentration as established by WHO 2011

<table>
<thead>
<tr>
<th>POPULATION</th>
<th>NORMAL HAEMOGLOBIN CONCENTRATION AT SEA LEVEL (in g/dL)</th>
</tr>
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<tbody>
<tr>
<td>Children (6-59 months of age)</td>
<td>11 or higher</td>
</tr>
<tr>
<td>Children (5-11-years of age)</td>
<td>11.5 or higher</td>
</tr>
<tr>
<td>Children (12-14 years of age)</td>
<td>12 or higher</td>
</tr>
<tr>
<td>Non-pregnant women (15 years of age and above)</td>
<td>12 or higher</td>
</tr>
<tr>
<td>Pregnant women</td>
<td>11 or higher</td>
</tr>
<tr>
<td>Men (15 years of age and above)</td>
<td>13 or higher</td>
</tr>
</tbody>
</table>
Table 1.2 Normal haemoglobin concentration as established by CDC\textsuperscript{24}

<table>
<thead>
<tr>
<th>POPULATION</th>
<th>NORMAL HAEMOGLOBIN CONCENTRATION AT SEA LEVEL (in g/dL)</th>
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<tbody>
<tr>
<td>Children</td>
<td></td>
</tr>
<tr>
<td>1-&lt;2 years of age</td>
<td>11.0 or higher</td>
</tr>
<tr>
<td>2-&lt;5 years of age</td>
<td>11.1 or higher</td>
</tr>
<tr>
<td>5-&lt;8 years of age</td>
<td>11.5 or higher</td>
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<tr>
<td>8-&lt;12 years of age</td>
<td>11.9 or higher</td>
</tr>
<tr>
<td>Men</td>
<td></td>
</tr>
<tr>
<td>12-&lt;15 years of age</td>
<td>12.5 or higher</td>
</tr>
<tr>
<td>15-&lt;18 years of age</td>
<td>13.3 or higher</td>
</tr>
<tr>
<td>&gt;=18 years of age</td>
<td>13.5 or higher</td>
</tr>
<tr>
<td>Non-pregnant women</td>
<td></td>
</tr>
<tr>
<td>12-&lt;15 years of age</td>
<td>11.8 or higher</td>
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<tr>
<td>15-&lt;18 years of age</td>
<td>12.0 or higher</td>
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<tr>
<td>&gt;=18 years of age</td>
<td>12.0 or higher</td>
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<tr>
<td>Pregnant women</td>
<td></td>
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<tr>
<td>First trimester</td>
<td>11.0 or higher</td>
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<tr>
<td>Second trimester</td>
<td>10.5 or higher</td>
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<tr>
<td>Third trimester</td>
<td>11.0 or higher</td>
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</tbody>
</table>
DENTAL CARIES AND ANAEMIA

The association between dental caries and anaemia has been well explored in the past but only in the paediatric population. Most of the studies have found a definite relationship between dental caries and various types of anaemia such as iron deficiency anaemia, sickle cell anaemia, thalassaemia and Fanconi anaemia.

DENTAL CARIES AND IRON DEFICIENCY ANAEMIA

Voluminous studies report the association of oral soft tissue pathologies with iron deficiency anaemia, but the association of dental hard tissue pathologies with iron deficiency anaemia is little explored, especially in an adult population.

Angular cheilitis and atrophic glossitis are the most commonly reported oral soft tissue manifestations of iron deficiency anaemia.\textsuperscript{25} Burning mouth sensation, altered taste sensation, numbness of the oral mucosa, lingual varicosity, oral lichen planus, oral candidiasis and xerostomia are some of the other reported oral manifestations in iron deficiency anaemia.\textsuperscript{26}

Iron plays a role in epithelial cell renewal, thereby its deficiency affects the oral mucosa which has a high turnover rate, resulting in such mucosal atrophy and alterations. Host immune response is also impaired in iron deficiency anaemia as iron plays an important role in normal functioning of lymphocytes, neutrophils\textsuperscript{27} and is a cofactor in various enzymatic reactions of the defence system,\textsuperscript{25} thereby increasing the risk of infections, especially in the oral cavity which is constantly exposed to a rich microbial flora.
The hard tissue disease of the oral region that is found to be in significant association with iron deficiency anaemia is dental caries especially in children as reported by the following authors.

Ramos-Gomez et al., in 2002\textsuperscript{28} studied the various demographic, behavioural, environmental and salivary bacterial factors and minerals in 63 children with early childhood caries, 23 children with incipient caries and 60 caries free children below 60 months of age at San Francisco. The cross-sectional study confirmed a significant association between ECC and salivary bacterial load, age, maternal education, and family income. Though the authors have claimed a lack of association between early childhood caries and anaemia, no mention about the assessment of anaemic status is made by the authors in their paper except for the figures on prevalence of iron deficiency in the study participants.

Clarke et al., in 2006\textsuperscript{29} evaluated the nutritional status of 56 children with early childhood caries, aged 2- to 6-years in a longitudinal study at Canada. Anthropometric measures (such as height, weight, triceps skin fold thickness, arm-circumference measurements) and blood parameters (such as serum albumin, haemoglobin, ferritin and mean corpuscular volume) were analysed. The study found significantly lower levels of haemoglobin and ferritin in children with early childhood caries and suggested severe early childhood caries to be a risk for iron deficiency anaemia in children.

Shaoul et al., in 2012\textsuperscript{5} evaluated the blood parameters for anaemia in 30 children aged 3- to 18-years with severe caries and 30 caries-free age and sex-matched children at Israel. The study revealed statistically significant differences in the values of haemoglobin, serum ferritin, serum iron levels, red cell distribution width and mean
corpuscular volume between the study group and control group. However, no significant differences were found in the levels of vitamin B12 and folic acid, thus implying that anaemia is certainly because of iron deficiency and not because of other causes. Based on their results, the authors claimed a strong association between iron deficiency anaemia and severe caries in children. To further validate their results, the authors evaluated the anaemia related blood parameters in the caries group, before and after 4 to 6 months of dental therapy without any form of supplement therapy to resolve iron deficiency anaemia. They found a significant improvement in the anaemic status of the participants with only dental therapy, thus strongly establishing the association between dental caries and iron deficiency anaemia.

Sadeghi et al., in 2012\(^3\) investigated the association of serum iron and ferritin levels with caries experience in 204 children of age ranging from 24- to 71-months at Iran. The authors confirmed a statistically significant inverse relationship between serum iron levels and decayed, extracted due to caries, and filled primary teeth (deft) index in the study cohort, while there was no association with serum ferritin levels.

Iranna Koppal et al., in 2013\(^3\) identified a significant association between iron deficiency anaemia and severe early childhood caries in a cross-sectional study at India. The authors evaluated the caries experience with decayed, extracted due to caries, and filled primary teeth (deft) index and the iron status with haemoglobin concentration, serum ferritin level and mean corpuscular volume in 60 children aged 2- to 6-years with 30 participants in the severe early childhood caries group and 30 in the control group.

Schroth et al., in 2013\(^5\) compared the iron status with blood parameters like haemoglobin, ferritin and mean corpuscular volume in 144 severe early childhood caries
participants and age-matched 122 caries-free controls of age less than 72 months at Canada. The case-control study revealed a 6 times higher risk of having iron deficiency anaemia in severe early childhood caries group than the caries-free group.

**Tang et al., in 2013** studied the relationship between dental caries and nutritional status in 101 participants with severe early childhood caries between 2- and 5-years of age at Taiwan. Anthropometric (body weight, height and body mass index) and biochemical investigations (clinical and anaemia related blood analysis) were performed to evaluate the nutritional status of the participants. Their results suggested a strong association between severe early childhood caries and iron deficiency anaemia with a 7.25 fold higher risk of iron deficiency anaemia in severe early childhood caries participants, thereby suggesting severe early childhood caries to be a risk factor for iron deficiency anaemia.

**Bansal et al., in 2016** investigated 30 children with severe early childhood caries and 30 caries-free children, aged 2- to 6-years at India. The study demonstrated a definite difference in haemoglobin concentration, mean corpuscular volume, and packed cell volume between the study groups and severe early childhood caries was recognised as a risk factor for iron deficiency anaemia.

**Nur et al., in 2016** studied the prevalence of iron deficiency anaemia in 160 children with severe early childhood caries, aged 2- to 6-years at Turkey and found a significant difference in the values of mean corpuscular volume, though there was no significant difference in haemoglobin and haematocrit values. The authors claimed severe early childhood caries to be a risk marker for iron deficiency anaemia.
Costa et al., in 2017\textsuperscript{34} reported the influence of iron deficiency anaemia on caries incidence in a sample of 121 pregnant women at Brazil. The authors recorded a higher risk of dental caries in the presence of iron deficiency anaemia and proposed the anaemia parameters in saliva to be a potential risk indicator for dental caries.

Venkatesh Babu et al., in 2017\textsuperscript{35} reported a significant inverse association between caries experience and iron status in a cross-sectional study at India. The study compared the decayed, extracted, filled tooth (deft) scores with serum iron and ferritin levels in 120 children aged 3-to 12-years.

Deane et al., in 2018\textsuperscript{36} investigated the combined vitamin D and anaemic status of 144 preschoolers aged below 72 months with severe early childhood caries and 122 caries free controls at Canada. The study revealed significantly lower haemoglobin levels and vitamin D levels in the severe early childhood caries group than the control group. The authors concluded on an increased prevalence of vitamin D deficiency and anaemia in severe early childhood caries children.

Bahdila et al., in 2019\textsuperscript{37} investigated the association between iron deficiency anaemia and dental caries in an animal model. The study induced iron deficiency anaemia in mice with iron-deficient cariogenic diet and the control group was fed on a similar cariogenic diet but iron-rich. The caries status in both the groups were compared and a significantly higher caries score was recorded in mice with iron deficiency anaemia than the control group, thereby suggesting a higher susceptibility of dental caries in iron deficiency anaemia.
Association between anaemia and dental caries in adults

DENTAL CARIES AND SICKLE CELL ANAEMIA

Okafor et al., in 1986\textsuperscript{38} compared the oral soft tissue and hard tissue manifestations in 37 Nigerian participants with sickle cell disease between 14- and 33-years of age with an age and sex matched control group of 20 participants without sickle cell disease. The authors reported a lower prevalence of dental caries in the disease group when compared with the control group; however the authors did not mention the statistical significance of this difference.

Laurence et al., in 2002\textsuperscript{39} at Washington DC and Baltimore investigated the prevalence of dental caries in 35 sickle cell anaemia participants, matched by age with 140 control participants, with a wide age range of 6- to 92-years. The DMFT score was found to be 21.1\% higher in the sickle cell anaemia group than the control group. The authors concluded a higher risk of caries susceptibility in sickle cell anaemia participants, which increased with increasing age.

Laurence et al., in 2006\textsuperscript{40} compared the DMFS scores in 102 African-American adult participants with sickle cell anaemia and 103 disease free cohort in a retrospective study and concluded that the participants with sickle cell anaemia had 3.6 times more decayed surfaces when compared with the control group.

Passos et al., in 2012\textsuperscript{41} investigated the oral manifestations of sickle cell disease in 190 Brazilian participants with sickle cell disease and a control cohort of 99 participants without sickle cell disease with age ranging from 16- to 68-years. The authors reported a significantly higher number of decayed surfaces in the study group than the control group.
**Fernandes et al., in 2015** compared the prevalence of dental caries in 106 Brazilian children diagnosed with sickle cell disease (SCD), between 8- to 14-years of age with 385 disease-free control participants. The study found a decreased caries experience in the children (8- to 10-years) with SCD, and no difference in the caries experience between the teens (11- to 14-years) with SCD when compared with their healthy controls. Free dental treatment to the paediatric SCD patients by the Brazilian government, water fluoridation, and the special care provided by the parents of SCD children were stated as the factors contributing to the decreased caries experience in the SCD study group.
DENTAL CARIES AND THALASSAEMIA

Kaplan et al., in 1964[43] studied the oral manifestations of thalassaemia major in 50 participants with the disease between 3- and 28-years of age at Philadelphia and claimed a higher prevalence rate of dental caries in the study group than that reported for the normal population of Philadelphia.

Siamopoulou-Mavridou et al., in 1992[44] investigated salivochromy of parotid saliva in relation to the caries and gingival status of 21 paediatric participants with thalassaemia major and 83 disease-free control group at Greece. The authors claimed a significant increase in the caries experience of thalassaemic participants than the control group.

Hattab et al., in 2001[45] investigated the prevalence of dental caries in 54 participants aged between 6- to 18-years with thalassaemia major and reported a 22.7% higher prevalence rate of dental caries in the study group than that reported for the normal disease free participants at Jordan.

Luglie et al., in 2002[46] compared the prevalence of dental caries and the oral hygiene status of 18 participants aged between 23- to 31-years with thalassaemia major with that of an age-and sex-matched control group of healthy participants at Italy. A higher prevalence rate of dental caries was found in thalassaemia major, though the reported difference was not statistically significant.

Al-Wahadni et al., in 2002[47] found a significantly higher prevalence of dental caries in 66 thalassemic participants when compared with 63 disease free control between 6-and 18-years of age at Jordan. The authors reported a two times higher DMFT/dmft indices in the thalassemic group than the control group.
Association between anaemia and dental caries in adults

Gomber et al., in 2006\textsuperscript{48} compared the prevalence of dental caries in 53 Indian thalassemic participants between 2- and 22-years of age with age-matched healthy controls. The study found a significantly higher DMFT and DMFS indices in thalassemic participants, thereby claiming a higher prevalence of dental caries in thalassemia.

Singh et al., in 2013\textsuperscript{49} studied the prevalence of dental caries, gingival and periodontal status in 250 participants with thalassaemia major, 250 participants with sickle cell anaemia and 250 healthy participants with all the study participants between 3- and 15-years of age at India. The study found a significantly increased caries prevalence in the thalassaemic participants followed by the sicklers than the control group of healthy participants.

Elangovan et al., in 2013\textsuperscript{50} investigated children with β thalassaemia major between 5-and 18-years of age and has recorded a higher caries experience (decayed, extracted, filled tooth score) in the 72 participants included in the study, although the authors have not mentioned whether the difference is statistically significant or not.

Kularatne et al., in 2018\textsuperscript{51} investigated the caries prevalence in 400 Sri Lankan participants with and without thalassemia and found a significantly increased caries prevalence in the control group than the thalassemia group.
DENTAL CARIES AND FANCONI ANAEMIA

Lyko et al., in 2016\textsuperscript{52} compared the caries experience, oral hygiene status and dental care level in 35 Brazilian participants with Fanconi anaemia and 35 age matched disease free control group between 6- to 18-years of age. The study found a higher caries experience (DMFT index) in the study group than the control group, though the difference was not statistically significant.
MATERIALS AND METHODS

The study was conducted in The Auto-Haematology and Biochemistry Laboratory associated with the Department of Oral and Maxillofacial Pathology and Microbiology, Tamil Nadu Government Dental College and Hospital, Chennai-600003. The study was approved by the Institutional Review Board, Reference No: 7/IRB/2017 dated 30.11.2017, Tamil Nadu Government Dental College and Hospital, Chennai 600 003.

The study design, study population, sampling method, sample size, inclusion criteria, exclusion criteria, operational definitions and data collection are as mentioned below:

1. STUDY DESIGN

The study was designed as a descriptive cross sectional survey.

2. STUDY POPULATION

The present study included adult patients (19- to 50-years of age) who were referred for laboratory blood investigations by various departments in Tamil Nadu Government Dental College and Hospital, Chennai-600 003.

3. SAMPLING METHOD

The study participants were recruited by simple random sampling method based on the inclusion and exclusion criteria.
4. SAMPLE SIZE

The sample size was calculated as

\[ \text{Sample size (n)} = \frac{Z^2 p (1-p)}{C^2} \]

\[ = 1.96^2 \times 0.5 \times (1-0.5)/0.05^2 \]

\[ = 384 \]

A confidence level of 95% was chosen for the study. A total of 403 participants were recruited for the present study.

5. INCLUSION CRITERIA

- Patients referred for laboratory blood investigations from other departments.
- Adult patients of age ranging from 19- to 50-years.
- Patients with normal mouth opening for intra-oral examination.

6. EXCLUSION CRITERIA

- Physically and mentally challenged patients.
- Completely edentulous patients.
- Medically compromised patients with conditions like hypertension, diabetes mellitus, and any other systemic diseases.
- Patients with other oral and jaw diseases.
- Pregnant patients.
7. OPERATIONAL DEFINITIONS

ANAEMIA

- The strict age- and gender- specific definitions established by the WHO for anaemia were used to categorise the participants into anaemic or non-anaemic group.\(^9\)
- Anaemia in female participants was defined as: haemoglobin concentration lesser than 12g/dL as per the WHO criteria.
- Female participants with haemoglobin concentration greater than or equal to 12g/dL were considered non-anaemic.
- Anaemia in male participants was defined as: haemoglobin concentration lesser than 13g/dL as per the WHO criteria.
- Male participants with haemoglobin concentration greater than or equal to 13g/dL were considered non-anaemic.
- Participants with MCV value lesser than 80fL were considered to have microcytic anaemia; MCV value between 80fL and 100fL were considered to have normocytic anaemia and MCV value greater than 100fL were considered to have macrocytic anaemia.
DENTAL CARIES

DMFT index as per the WHO criteria was used to record the caries experience of the participants included in the study. Clinical examination using an artificial source of light, mouth mirror and dental probe was done to determine the DMFT score of the study participants.

- The criteria used for defining D, M, and F component are mentioned below –

- D-component was defined to include all the permanent teeth with -
  - Any carious lesion that had a catch when probed with the dental explorer.
  - Open cavitated lesions.
  - Temporary restorations.
  - Secondary caries following restoration.

- M-component was defined to include all the permanent teeth which –
  - Are missing due to a history of dental caries.
  - Are grossly decayed and were indicated for extraction.
  - Are missing due to reasons other than caries like extracted for orthodontic purposes, trauma, congenitally missing, impacted teeth were not included.
• F-component was defined to include all the permanent teeth with –
  o Any restoration following the excavation of dental caries.

• Calculation of the index –
  o The D, M and F scores were added to give a final DMFT score
  o The DMFT score for an individual ranged from 0 to 32, as third molars were also included.
8. DATA COLLECTION

- The investigations and dental examinations were carried out at The Auto-Haematology and Biochemistry Laboratory of the Department of Oral and Maxillofacial Pathology and Microbiology, Tamil Nadu Government Dental College and Hospital, Chennai-600003 for the patients who were referred for blood investigations from other departments.

- Baseline characteristics like age, sex and a short medical and dental history was obtained from the study participants who satisfied the inclusion criteria after obtaining their written informed consent.

- The haemoglobin concentration and mean corpuscular volume (MCV) of the study participants were estimated with a computerised Sysmex XN-1000 haematology analyser (Sysmex Corporation, Kobe, Japan) by trained laboratory technicians in The Auto-Haematology and Biochemistry Laboratory of the Department of Oral and Maxillofacial Pathology and Microbiology, Tamil Nadu Government Dental College and Hospital, Chennai-600003.

- The haemoglobin concentration and MCV of the study participants were recorded. The study sample was divided into two groups namely – Anaemic group and Non-anaemic group. Female participants with haemoglobin level < 12 g/dL and male participants with haemoglobin level < 13 g/dL were included in the anaemic group. Female participants with haemoglobin level ≥ 12 g/dL and male participants with haemoglobin level ≥ 13 g/dL were included in the non-anaemic group.
• Anaemia was classified based on the MCV values as microcytic, normocytic and macrocytic.

• Each group was stratified according to ages in decade as 19-29-years, 30-40-years and 41-50-years in order to eliminate the confounding effect of age on dental caries and anaemia.

• Dental examination was carried out for the study participants of both the groups under an artificial source of light, mouth mirror and dental probe by a single student investigator and DMFT index as per WHO criteria was recorded.

• The data thus obtained were entered in a pre-designed clinical assessment form for each study participant and the records were maintained.

• The data were entered in an MS-Excel worksheet (Microsoft office, Windows 7 Home Basic) and submitted for statistical analysis.
STATISTICAL ANALYSIS

The Normality tests Kolmogorov-Smirnov and Shapiro-Wilks tests results revealed that haemoglobin% followed normal distribution and DMFT did not follow normal distribution. Therefore, to analyse the data both parametric and non parametric methods were applied. To compare the mean haemoglobin (%) values between groups independent samples t-test was applied. To compare proportions between groups Chi-Square test was applied, if any expected cell frequency was less than five then Fisher’s exact test was used. Risk ratio with 95% CI was calculated. To compare DMFT values between groups Mann Whitney U test was used. To analyse the data SPSS (IBM SPSS Statistics for Windows, Version 23.0, Armonk, NY: IBM Corp. Released 2015) was used. Significance level was fixed as 5% (α = 0.05).
RESULTS

A total of 403 participants were included in the study with 200 participants in the anaemic group and 203 participants in the non-anaemic group. The haemoglobin concentration, MCV and DMFT score were recorded. The haemoglobin concentration and DMFT scores were submitted for statistical analysis. The type of anaemia recorded in the study participants was determined by the MCV values as microcytic, normocytic or macrocytic.

The age and gender distribution were studied and tabulated (Table-2 and 3). The study included 138 participants in 19-29-years age-group, 149 participants in 30-40-years age group and 116 participants in 41-50-years age-group.

Number of participants and their age in decades

The results revealed that the age of the study participants ranged from 19-years to 50-years for both the groups.

The anaemic group comprised of 200 patients (20 – 50-years; female 189 (20-50-years with a mean of 34-years), males 11 (range 25-48-years with a mean of 40-years).

The non-anaemic group comprised of 203 patients (range 19-50-years; female 72 (19-50-years with a mean of 35-years), males 131 (range 20-50-years with a mean age of 34-years).

Haemoglobin concentration in the participants

In anaemic group, the haemoglobin ranged from 4.6 g/dl to 11.9 g/dl in females (mean 10.39 g/dl) and 6.0 g/dl to 12.9 g/dl (11.54 g/dl) in males.
In non-anaemic group, the haemoglobin ranged from 12.1 g/dl to 17 g/dl (mean 13.14 g/dl) in females and 13.2 g/dl to 20.4 g/dl (mean 14.96 g/dl) in males.

**MCV in the participants**

In anaemic group, the MCV values ranged from 54.7 fL to 98.3 fL in females with a mean of 79.75 fL and the MCV values ranged from 67 fL to 100.8 fL in males with a mean of 82.86 fL. A mean MCV of 79.924 fL was obtained for both the genders with a range of 54.7 to 100.8 fL.

In non-anaemic group, the MCV values ranged from 72.1 fL to 97.3 fL in females with a mean of 85.41 fL and the MCV values ranged from 71.5 fL to 103.7 fL in males with a mean of 87.65 fL. A mean MCV of 86.86 fL was obtained for both the genders with a range of 71.5 to 103.7 fL.

**DMFT index in the participants**

In anaemic group, the mean DMFT score with standard deviation was obtained as 5.20±4.97 for both the genders, whereas it was 4.00±3.16 for male participants and 5.27±5.05 for female participants. (Table-4)

In non-anaemic group, the mean DMFT score with standard deviation was 3.48±3.10 for both the genders, whereas it was 3.30±3.16 for male participants and 3.81±2.98 for female participants. (Table-4)

The mean DMFT scores of the anaemic and non-anaemic groups were compared to analyse the relationship between dental caries and anaemia (Figure 3). The p-value obtained using Mann Whitney test was <0.001 which signifies a statistically strong
association between the DMFT scores and haemoglobin levels of the study groups (Table-5).

The p-value was calculated for each age strata so as to eliminate the confounding effect of age on dental caries and anaemia and was found to be 0.006 for 19-29-years age group; 0.038 for 30-40-years age group; and 41-50-years age group, thereby suggesting a statistically significant association between DMFT scores and haemoglobin levels in all the three age groups of adult patients (Table-6).

The calculation of relative risk shows that anaemic participants are at a 1.090 fold higher risk of being affected by dental caries when compared with non-anaemic participants (Table-7.1, 7.2 and 7.3). Likewise, anaemic participants with adult rampant caries (DMFT score≥8) were shown to be at a 1.210 fold higher risk than the non-anaemic participants (Table-8.1, 8.2 and 8.3).

The results of the statistical analysis, thus suggests a statistically significant association between dental caries (DMFT scores) and anaemic status (haemoglobin levels) in adult participants included in the study.
Table -2 Distribution of the gender in the study group according to age

<table>
<thead>
<tr>
<th>Gender</th>
<th>Age Group</th>
<th>19-29-years</th>
<th>30-40-years</th>
<th>41-50-years</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>Male</td>
<td>53</td>
<td>37.3%</td>
<td>41</td>
<td>28.9%</td>
<td>48</td>
</tr>
<tr>
<td>Female</td>
<td>85</td>
<td>32.6%</td>
<td>108</td>
<td>41.4%</td>
<td>68</td>
</tr>
<tr>
<td>Total</td>
<td>138</td>
<td>34.2%</td>
<td>149</td>
<td>37.0%</td>
<td>116</td>
</tr>
</tbody>
</table>

Table-3 Distribution of the gender in the two study groups according to age

<table>
<thead>
<tr>
<th>Group</th>
<th>Gender</th>
<th>19-29-years</th>
<th>30-40-years</th>
<th>41-50-years</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>Anaemic</td>
<td>Male</td>
<td>3</td>
<td>27.3%</td>
<td>1</td>
<td>9.1%</td>
</tr>
<tr>
<td>Group</td>
<td>Female</td>
<td>67</td>
<td>35.4%</td>
<td>75</td>
<td>39.7%</td>
</tr>
<tr>
<td>Total</td>
<td>70</td>
<td>35.0%</td>
<td>76</td>
<td>38.0%</td>
<td>54</td>
</tr>
<tr>
<td>Non-</td>
<td>Male</td>
<td>50</td>
<td>38.2%</td>
<td>40</td>
<td>30.5%</td>
</tr>
<tr>
<td>Anaemic</td>
<td>Female</td>
<td>18</td>
<td>25.0%</td>
<td>33</td>
<td>45.8%</td>
</tr>
<tr>
<td>Group</td>
<td>Total</td>
<td>68</td>
<td>33.5%</td>
<td>73</td>
<td>36.0%</td>
</tr>
</tbody>
</table>
Boxplot comparing DMFT scores between anaemic and non-anaemic groups.
Association between anaemia and dental caries in adults

**Table-4** The mean DMFT scores among the anaemic and non-anaemic groups.

<table>
<thead>
<tr>
<th>DMFT</th>
<th>Anaemic group</th>
<th>Non-Anaemic group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>N</td>
<td>11</td>
<td>189</td>
</tr>
<tr>
<td>Mean</td>
<td>4.00</td>
<td>5.27</td>
</tr>
<tr>
<td>Std. Dev.</td>
<td>3.16</td>
<td>5.05</td>
</tr>
<tr>
<td>Median</td>
<td>2.0</td>
<td>4.0</td>
</tr>
<tr>
<td>1st Quartile</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>3rd Quartile</td>
<td>5.0</td>
<td>8.0</td>
</tr>
</tbody>
</table>

**Table-5** Mann-Whitney U Test to compare DMFT scores between anaemic and non-anaemic groups

<table>
<thead>
<tr>
<th></th>
<th>Z-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>0.957</td>
<td>0.339</td>
</tr>
<tr>
<td>Female</td>
<td>1.735</td>
<td>0.083</td>
</tr>
<tr>
<td>Total</td>
<td>3.551</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Table -6 Mann-Whitney U Test to compare DMFT scores between anaemic and non-anaemic groups with age stratification.

<table>
<thead>
<tr>
<th>Age Group</th>
<th>DMFT</th>
<th>Groups</th>
<th>Z-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Anaemic group</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Non-Aneamic group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19-29-years</td>
<td>N</td>
<td>70</td>
<td>2.763</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>3.77</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.57</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Std. Dev.</td>
<td>3.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.91</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>3.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1st Quartile</td>
<td>2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3rd Quartile</td>
<td>6.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-40-years</td>
<td>N</td>
<td>76</td>
<td>2.074</td>
<td>0.038</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>5.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.47</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Std. Dev.</td>
<td>5.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.66</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>4.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1st Quartile</td>
<td>2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3rd Quartile</td>
<td>8.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>41-50-years</td>
<td>N</td>
<td>54</td>
<td>1.996</td>
<td>0.046</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>6.81</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.48</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Std. Dev.</td>
<td>6.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>5.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1st Quartile</td>
<td>3.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3rd Quartile</td>
<td>9.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table-7.1 Prevalence of dental caries in anaemic and non-anaemic groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Dental Caries</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No (DMFT=0)</td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>Yes (DMFT≥1)</td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>Anaemic group</td>
<td></td>
<td>26</td>
<td>13.0%</td>
<td>174</td>
<td>87.0%</td>
</tr>
<tr>
<td>Non-Aナemic group</td>
<td></td>
<td>41</td>
<td>20.2%</td>
<td>162</td>
<td>79.8%</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>67</td>
<td>16.6%</td>
<td>336</td>
<td>83.4%</td>
</tr>
</tbody>
</table>

Table-7.2 Pearson Chi-Square test to compare the prevalence of dental caries between anaemic and non-anaemic groups

<table>
<thead>
<tr>
<th>Chi-Square Tests</th>
<th>Value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson Chi-Square</td>
<td>3.765</td>
<td>0.052</td>
</tr>
</tbody>
</table>

Table-7.3 Risk estimate of dental caries in anaemic and non-anaemic groups.

<table>
<thead>
<tr>
<th>Risk Estimate</th>
<th>Value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>LB</td>
</tr>
<tr>
<td></td>
<td></td>
<td>UB</td>
</tr>
<tr>
<td>Relative Risk</td>
<td>1.090</td>
<td>0.999</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.190</td>
</tr>
</tbody>
</table>
Table-8.1 Prevalence of adult rampant caries in anaemic and non-anaemic groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Adult Rampant Caries</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No (DMFT score&lt;7)</td>
<td>Yes (DMFT score≥8)</td>
<td>Total</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>Anaemic</td>
<td>150</td>
<td>75.0%</td>
<td>50</td>
</tr>
<tr>
<td>Non-Aneamic</td>
<td>180</td>
<td>88.7%</td>
<td>23</td>
</tr>
<tr>
<td>Total</td>
<td>330</td>
<td>81.9%</td>
<td>73</td>
</tr>
</tbody>
</table>

Table-8.2 Pearson Chi-Square test to compare the prevalence of adult rampant caries between anaemic and non-anaemic groups

<table>
<thead>
<tr>
<th>Chi-Square Tests</th>
<th>Value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson Chi-Square</td>
<td>12.692</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table -8.3 Risk estimate of adult rampant caries in anaemic and non-anaemic groups.

<table>
<thead>
<tr>
<th>Risk Estimate</th>
<th>Value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>LB</td>
</tr>
<tr>
<td>Relative Risk</td>
<td>1.21</td>
<td>1.40</td>
</tr>
</tbody>
</table>
DISCUSSION

Dental caries is a complex microbial disease with many known and unknown contributing factors. An extensive understanding of the pathogenesis of any disease forms the basis for its successful management and prevention strategies. The present investigation is an attempt to explore the possible role of anaemia as a risk factor in the etiology of dental caries.

The present investigation is a cross-sectional survey that compared the DMFT scores in anaemic and non-anaemic participants between 19- to 50-years of age, in order to evaluate the association between dental caries and anaemia. As the association between dental caries and anaemia has already been well established in the paediatric age group,\textsuperscript{5-7} this study investigated only the adult participants between 19- to 50- years of age. A recent study has claimed dental caries in permanent dentition to be the most prevalent disease worldwide in 2010, whereas dental caries in deciduous dentition ranked the 10\textsuperscript{th} most prevalent condition in the world,\textsuperscript{54} thus adding significance to the present investigation.

The strict age- and gender-specific definitions for anaemia as established by the WHO have been used to identify the study group as anaemic and non-anaemic group. The DMFT index of the study participants has also been recorded as per the WHO criteria. The present investigation therefore is based on the WHO standards, thus making the study results pertinent to the global population as well, though only a specific population of the Indian subcontinent was investigated.
The WHO recognises haemoglobin concentration as the most reliable indicator of anaemia at the community level, though the haemoglobin concentrations alone cannot determine the type of anaemia.

Our study revealed that among the 403 adult patients, the anaemic patients had a mean Hb concentration of 10.47±1.64 g/dL and a mean DMFT score of 5.20±4.97, whereas the mean Hb concentration was 14.32±1.39 g/dL and the mean DMFT score was 3.48±3.10 in the non-anaemic patients. These data thus suggest that lower Hb concentrations are related to higher DMFT scores, demonstrating an inverse association which was found to be statistically significant (p-value < 0.001). Therefore, it is surmised that patients with anaemia are found at a higher risk of developing dental caries than non-anaemic patients.

The present study has attempted to classify the type of anaemia based on the MCV values into microcytic, normocytic or macrocytic type. Among the various types of anaemia, iron deficiency anaemia remains the most important and the most common form of anaemia which is microcytic as well as normocytic in nature followed by anaemia of chronic inflammation. Of the anaemic patients, about 60% (120/200) presented with normocytic anaemia (MCV = 80 to 100 fL) while the remaining 40% (80/200) had microcytic anaemia (MCV<80fL). In addition, none of the study participants reported with a medical history relevant to other forms of anaemia such as sickle cell anaemia, thalassaemia, and Fanconi anaemia, as these forms of anaemia most often manifests in the childhood itself. Therefore, lower Hb concentrations along with lower or normal MCV values in anaemic patients of the present study indicate either iron deficiency anaemia or anaemia of chronic inflammation.
The literature has enormous evidence confirming the association of soft tissue pathologies of the oral cavity with iron deficiency anaemia, but the association of hard tissue pathologies of the oral cavity with iron deficiency anaemia is little explored, especially in an adult population.

The oral hard tissue manifestation which is found to be significantly associated with iron deficiency anaemia is childhood dental caries. Various reports in the previous literature have established the relationship between early childhood caries and iron deficiency anaemia in paediatric population. Although a previous study has claimed a lack of association between early childhood caries and anaemia, the assessment of anaemic status has not been described by the authors in their paper except for the figures on prevalence of iron deficiency in their study participants. Significant evidence suggests an improvement in the anaemic status of the participants with iron deficiency anaemia following caries management, thus establishing a strong association between dental caries and iron deficiency anaemia. The increased susceptibility to dental caries in iron deficiency anaemia has also been proved in animal models.

The increased prevalence of dental caries in participants with iron deficiency anaemia is mainly attributed to the salivary gland hypo-functioning which results in reduced salivary secretion and buffering capacity. Animal studies have shown a reduced salivary secretion in iron deficiency anaemia, thus making the environment conducive for dental caries.

Iron is required for the normal differentiation and functioning of all cells and their enzyme systems. The intra-cellular iron takes part in the synthesis of haemoproteins and other iron containing proteins which are essential for normal cellular house-keeping.
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Iron also has a protective role against the free radical damage of the lipid membranes, proteins and nucleic acids, though an excess of cellular iron can have the opposite effects. Cellular growth arrest and cell-death have been reported with iron deficiency.\textsuperscript{58} The salivary epithelial cells are no exception to these adverse effects of iron deficiency, thus resulting in salivary gland hypo-function.

Iron supplementation has been known to increase the salivation in iron deficient patients which certifies the role of iron in normal salivary gland functioning, though contradicting results have also been reported.\textsuperscript{59,60}

The higher susceptibility of anaemic participants to dental caries is also attributed to the local anti-cariogenic effects of iron.\textsuperscript{56,61,62,63} Saliva has been known to play an important role in maintaining the integrity of the dental hard tissues by regulating its mineral homeostasis and thereby preventing the dissolution of hydroxyapatite from the dental hard tissues.\textsuperscript{60} Iron has a local action in preventing demineralisation of the calcified structures of the tooth by forming an acid-resistant protective layer of hydrous iron-oxides, which has a strong affinity to the organic constituents of the enamel and dentin.\textsuperscript{56,64}

Iron plays a role in remineralisation of the early carious lesions as well, by nucleating the calcium and phosphate ions from saliva.\textsuperscript{56} A higher concentration of iron is recorded in remineralised carious lesions than the intact surface layers of the teeth which further warrants the cariostatic role of iron.\textsuperscript{64}

The anti-microbial action and buffering capacity of the salivary proteins also have a protective role against dental caries.\textsuperscript{60} Normal salivary iron content ranges from 0.1 to 1.0 $\mu$M. Reduced iron concentration in the saliva creates an unfavourable micro-
environment for the streptococcus mutans (S.mutans) which is the pioneer organism in the pathogenesis of dental caries. S.mutans resists this stress by aggregating and evolving into an adherent bio-film which is conducive for dental caries. On the other hand, S.mutans aggregation was inhibited in iron rich saliva.\textsuperscript{65}

In-vitro studies have reported an inhibition of S.mutans with iron concentrations greater than 5mM.\textsuperscript{62} Salivary peroxidise which reduces the growth of S.mutans, is a haem-containing enzyme, which requires iron for its normal functioning. In iron deficiency anaemia, this enzyme is shown to be defective,\textsuperscript{57} thereby increasing the microbial load. Therefore an inverse relationship exists between the salivary iron concentration and the bacterial aggregation efficiency,\textsuperscript{65} which supports our speculation that iron deficiency anaemia could be a potential risk factor for dental caries.

A fall in acid synthesis by the salivary flora has been reported in the presence of metal ions such as zinc, aluminium and iron,\textsuperscript{66} thus helps maintaining a higher resting pH in the dental plaque.\textsuperscript{61,62} A rapid restoration of the plaque pH to normal levels following an acidogenic challenge has been reported in-vitro under the influence of iron despite the absence of salivary buffers, which further validates the action of iron in halting the demineralisation cascade of dental caries.\textsuperscript{61,62}

Dental plaque contains iron in notable amounts and are found to inhibit the thiol-group containing enzymes, F-ATPase and glucosyl-transferases, synthesized by S.mutans which are necessary for the fermentation of carbohydrates to produce the acids, thus defending against the carious process.\textsuperscript{34,56,62,67} The displacement of magnesium ions and the oxidation of thiol-group by iron cations are the proposed mechanisms behind this enzymatic inhibition.\textsuperscript{66} Synthesis of extra-cellular polysaccharides by S.mutans, which is
an important virulence factor, is also shown to be reduced by iron, thus adding to its anti-cariogenic activity.\textsuperscript{67}

Systemic as well as topical use of iron supplements has been found to reduce the caries susceptibility in various in-vitro and in-vivo studies.\textsuperscript{56,64} Topical rinse with 20mM ferric chloride solution has shown a fall in acid formation in human plaque,\textsuperscript{66} thus advocating the efficiency of iron as an anti-cariogenic agent like fluorides.

Studies in the literature have also favoured the theory of “anaemia of chronic disease” being caused by the immune activation following a chronic infectious disease such as dental caries and its sequel, especially its rampant forms.\textsuperscript{6} Various acute and chronic infections, malignancies, and inflammatory disorders lead to a cascade of events that results in anaemia, termed as “anaemia of chronic disease or anaemia of inflammation.”\textsuperscript{68} which is ranked the second most common type of anaemia after iron deficiency anaemia.\textsuperscript{69}

Iron sequestration and reduced erythropoiesis are proposed to be the main mechanisms underlying the pathophysiology of anaemia of inflammation. The marrow macrophages store iron which is transferred to erythroid precursors for heme synthesis during the process of erythropoiesis.\textsuperscript{70} This transfer of iron is inhibited by hepcidin, a protein secreted by hepatocytes and immune cells in response to infection and inflammation,\textsuperscript{68} thereby sequestering the iron molecules away from the erythroid precursors and arresting erythropoiesis, thus culminating in anaemia.\textsuperscript{71}

Interleukin-6 and interleukin-1β are the major inflammatory mediators that influence the erythrocytes by promoting the expression of hepcidin protein thereby inhibiting erythropoiesis. The lipopolysaccharides released by some bacteria during
infections also induce the secretion of interleukin-6 by macrophages which inhibit erythropoiesis via hepcidin.⁶⁸,⁷⁰

Inflammatory mediators such as interferon-γ influence the erythroid precursors directly by increasing their resistance to erythropoietin, which arrests further proliferation and differentiation or induces apoptosis in erythroid precursors.⁶⁸ The synthesis of erythropoietin by renal cells is also reduced by inflammatory mediators.⁶⁹ Therefore, there exists a broad spectrum of mechanisms underlying the pathogenesis of anaemia of inflammation.

The bacterial toxins and other bio-active molecules released during demineralisation in the course of dental caries provoke an inflammatory response in the pulp. Such a pulpal inflammation is often chronic in nature and persists even after the removal of the noxious stimuli. Recent studies on cellular and molecular mechanisms involved in the pathogenesis of dental caries have substantiated the role of inflammatory mediators being secreted by the pulpal cells in response to a cariogenic insult.⁷²

In-vivo and in-vitro studies have demonstrated the up-regulation of interleukin-6, a pro-inflammatory mediator in the diseased pulp.⁷² In addition to interleukin-6, interleukin-1β and interferon-γ are also reported to be secreted by the odontoblast-like cells and immune cells of the dental pulp following a carious insult.⁷³ Therefore, an up-regulation of the inflammatory cytokines has been described in dental caries which are also the key molecules underlying the pathogenesis of anaemia of inflammation.
STUDY LIMITATIONS

Proximal surface caries could have been missed as radiographs were not taken for the diagnosis of dental caries. The income level which is another confounding factor for dental caries and anaemia could not be evaluated, but as the study participants were recruited from an almost similar socio-economic background, the confounding bias by income level is less likely. Despite the limitations, the present study is remarkable as the association between anaemia and dental caries in an adult population has been demonstrated for the first time and would encourage similar studies in future to explore the true causal relationship between anaemia and dental caries.
SUMMARY AND CONCLUSION

In summary, the present study compared the caries status in anaemic and non-anaemic participants aged between 19- to 50-years, in order to determine the association between anaemia and dental caries in adults. Statistical analysis revealed a significant association between dental caries and anaemia.

There is no previous data in so far as the association between dental caries and anaemia in adult patients to make valid comparison with the present study. Thus, our findings shall be regarded as the starting point for comparison of future studies in adult patients.
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