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Type 1 Diabetes Mellitus And Associated Morphological Alterations In The Permanent Dentition –A Cross Sectional Study

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ABSTRACT:

Background: *Type 1 Diabetes Mellitus (T1DM) is an endocrinal disorder caused by the autoimmune destruction of beta cells of pancreas. It occurs more commonly in younger age group and hence termed as juvenile diabetes. Odontogenesis is the complex process of tooth formation involving genetic, epigenetic and environmental factors to control the interaction between epithelium and ectomesenchyme. Our study aimed to know the effect of T1DM on the odontogenic apparatus.* ***Methodology and Results:*** *39 T1DM patients were taken for the study and their dental morphology was recorded using alginate impression material. Out of 39 patients, 8 were aged less than or equal to 6 years and presented with only deciduous dentition. The remaining 31 T1DM (N=31) patients were included in the study and the morphology of their permanent dentition was studied. Gender matched subjects less than 20 years of age without diabetes mellitus were taken as controls and their morphology was recorded. On comparison, 61% (n=19) of the T1DM patients showed dental anomalies, in contrast to 12.9 % (n=4) of the controls. Sixth cusp in mandibular molars, multiple prominent grooves, pits and fissures on the occlusal surfaces of molars and premolars, enamel hypoplasia were the commonly observed anomalies.* ***Conclusions:*** *T1DM influence odontogenesis through systemic effects like hyperglycemia, chronic inflammation and vascular changes. Further studies at genetic level can shed more light on this topic.*

Key words: *Morphological alterations, Odontogenesis, Type 1 Diabetes Mellitus,Tuberculumintermedium, Tuberculumsextum*

INTRODUCTION:

Diabetes mellitus is a metabolic disorder (Diabetes – meaning siphon in Greek-"to pass", mellitus – Latin word meaning "sweet"), in which the body either does not produce enough insulin or does not respond normally to insulin or both, thereby causing increased blood glucose levels[1]. According to etiology, Diabetes mellitus (DM) is classified into four types, by the World Health Organisation (1998), including type 1, type 2, type 3 and type 4. Type 1 is caused by beta cell destruction, thereby causing absolute insulin deficiency. It is more commonly diagnosed in younger age group of 10-14 years and constitutes about 5-15% of the DM cases.Type 2 occurs in relatively older age group and is caused by some degree of insulin resistance. Type 2 DM accounts for 75-80% of the DM cases. Type 2 DM is rightly termed as the disease of the millennium, as it is affecting the individuals worldwide in an

alarmingly increasing rate(upto 529 millions of people in 2021[2]). Sedentary life style and change in the food habit is attributed to this tremendous change in the increasing rate. Type 3 DM (forming 5% of DM cases), is caused by other specific reasons, such as genetic cause, diseases of the pancreas, endocrinopathies, drug or chemical induced, due to infections,etc., Gestational diabetes is classified as the type 4 and it disappears after delivery, but these mothers are prone for type 2 DM in later stages of life. Each subtype has different etiologies, different presentations and different treatments[1]. Diabetes mellitus affects all the organs of the body by its persistent hyperglycemic effect and its effect on odontogenic apparatus is not yet studied. Our study aims to study the effect of type 1 diabetes mellitus (T1DM)on the permanent dentition of human subjects.

METHODOLOGY:

After obtaining Ethical Committee approval, this study was conducted on T1DM patients reporting to a private Diabetology and Endocrinology centre. This non-invasive study involves the clinical examination of the oral cavity of T1DM patients.Informed consent was obtained from the patients, questionnaire was filled and recording of their maxillary and mandibular impressions were done using alginate impression material.The questionnaire includes the details abouttheir initial clinical signs and symptoms, disease duration, medications taken, familial history of DM, blood sugar level on the day of examination. Their maxillary and mandibular impressions were recorded and photographs of the intraoral cavity were also taken.

The morphology of the dentition recorded from T1DM diabetes mellitus patients is then compared with the morphology of the dentition obtained from gender matched controls without diabetes mellitus. The standards given in Wheeler's text book were taken for reference. The morphological alterations observed were tabulated and statistical analysis was done (Table 1).

RESULTS:

Our study included 39 patients reporting to the Diabetology centre from various parts of Tamil Nadu, Pondicherry and Kerala, of which 24 were females and 15 were males. The age group of the patients included in the study ranged from 4 years to 38 years, with average being 14.17 years. The age of diagnosis of diabetes mellitus for these patients ranged from 6 months to 34 years, with average being 9.1 years. Their initial clinical signs and symptoms, before the diagnosis of T1DM was frequent urination, sudden weight loss, sudden emergency admission due to diabetic ketoacidosis. Currently, the patients are under regular treatment protocol. They are taking insulin, either through pen or pump to maintain optimal glycemic control.

Of these 39 patients, 8 patients were aged 6 or less than 6 years, presenting only with deciduous dentition. When their deciduous dentition was evaluated for morphological alterations, one subject was presenting with 6 cusps in 75.

The morphological alterations in permanent dentition were evaluated in the remaining 31 patients (N=31) (Table1).19 patients out of remaining 31 patients, were presenting with characteristic morphological changes in the permanent dentition. The most commonly observed alteration was the presence of sixth cusp in mandibular first molar, known as tuberculumintermedium (presence of extra sixth cusp in the lingual side, in between the two lingual cusps) or tuberculumsextum (presence of sixth cusp on lingual side, in the distal end) (Figure 1). Two patients had mandibular first molars with 4 cusps. The other alterations noted were large maxillary molars with two supernumerary cusps on both right and left side (Figure 2), Talon's cusp, peg laterals, absence of lateral incisors (Figure 3), presence of 4 to 5 cusps in mandibular second premolars (Figure 4B), presence of mount like extra lobes in occlusal surface and central pit regions of mandibular molars (Figure 4B). A 14 year old male patient, presented with kidney transplantation history before 6 months, due to target organ damage caused by hyperglycemia. His oral presentations were characteristic, where both the mandibular first molars were

presenting with six cusps. The cusps were sharp and showing severe enamel hypoplasia (Figure 4A) with attrition. Many patients presented with presence of numerous prominent extra pits, grooves and fissures on the occlusal surface of maxillary and mandibular molars and premolars, which were giving them micromorphological changes, without forming extra cusps. Other notable morphological change presented was in a female patient, who was having maxillary right central incisor with mesial angle being more rounded and shorter than distal side.

Other than morphological alterations, dental caries (DC) was observed more commonly in these T1DM patients. The severity of DC ranged from small pit caries to root stump exposure, where 5 patients showed root stumps.Malocclusion and varying severity of attrition was noted.Also, two of them showed retained deciduous dentition. Retained mandibular deciduous incisors (at 14 years of age), multiple retained deciduous teeth (at 18 years), and delay in eruption of their permanent dentition were seen.

In the control group, 31 gender matched subjects without diabetes mellitus were evaluated. The age of control group was less than 20 years. When the control group was evaluated for morphological changes, 4 persons out of 31 were showing dental anomalies (12.9%) and 27 subjects were without any morphological alterations in the dentition. The dental anomalies observed were tabulated in table 1. T1DM group showed statistically significant difference in occurrence of dental anomalies. No retained deciduous was observed in control group. Dental caries was observed in two controls and those caries were involving only occlusal surface and not severe upto the level of root stump formation as seen in the T1DM group.

Table 1: Morphological changes observed in the permanent dentition of type 1diabetes mellitus patients

S.No	Six cusps in mandibular molars	Prominent maxillary molars with extra cusp	Extra cusps in mandibular premolars	Talon's cusp	Prominent extra pits, grooves and fissures on occlusal surface of molars and premolars	Extra lobes on the occlusal surface of molars	Other morphological changes	Enamel hypoplasia	Total number of patients showing morphological changes
T1DM patients (N=31)	9 (29%)	3(9.6%)	3(9.6%)	3 (9.6%)	10 (32.2%)	3(9.6%)	6(19.35%)	4(13%)	19(61%)
Control group	2 (6.4%)	1 (3.2%)	0	1 (3.2%)	1(3.2%)	0		1(3.2%)	4(12.9%) P<0.001

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Figure 1: 1A. Presence of tuberculum sextum in right and left mandibular molars 1B. Presence of tuberculum intermedium in mandibular first molar

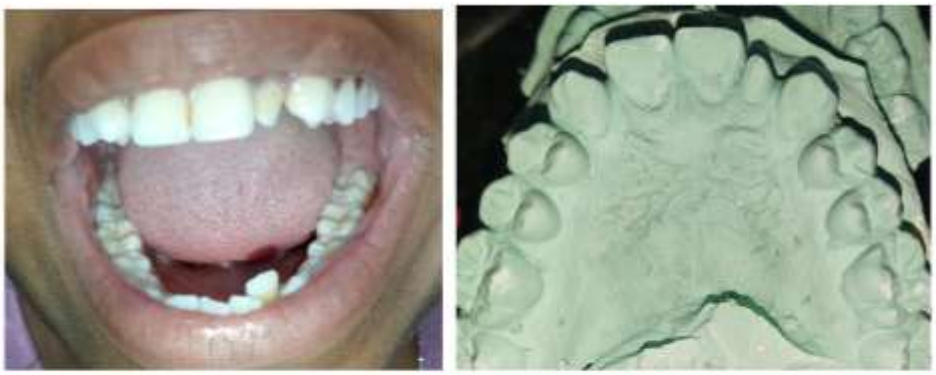

Figure 3: Maxillary right lateral incisor is missing and left lateral incisor is peg shaped. Clinical photograph and maxillary impression of the same patient

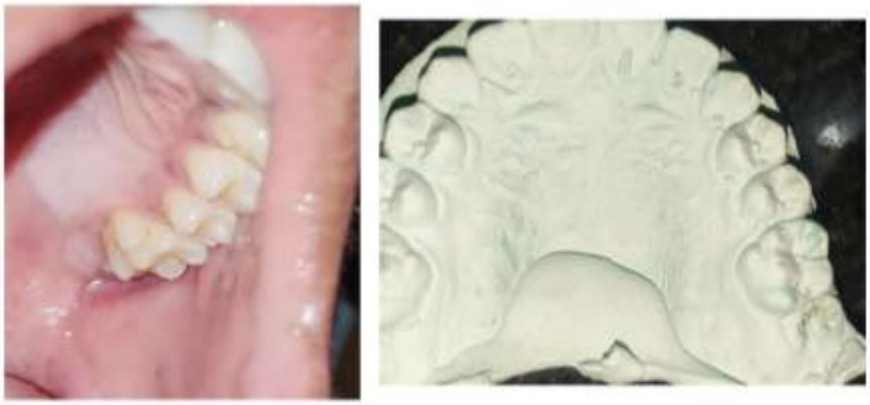

Figure 2: Maxillary first molar showing four cusps, and also two prominent cusps (two Cusps of Carabelli??). Clinical photograph and maxillary impression of the same patient

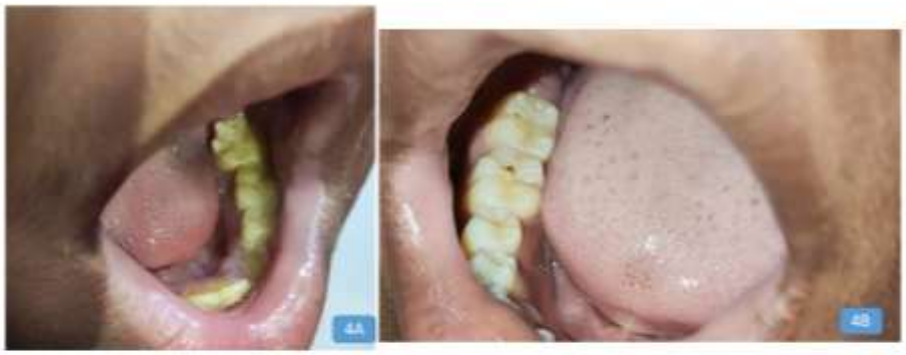

Figure 4: 4A. 14 year old male patient showing 3 sharp lingual cusps in mandibular left first molar. Severe attrition and enamel hypoplasia is also noted. 4B. 13 year old male patient showing more than 3 lingual cusps in mandibular right second premolar and lobe like elevation in the central pit region of mandibular right first molar

DISCUSSION:

Morphological alterations in the dentition of diabetes mellitus is being studied by us for many years and the various underlying mechanisms behind these alterations, including chronic low grade inflammation and hyperglycemiaare elucidated in the articles published before[3,4,5,6,7]. In this study, around 61% of T1DM were showing morphological alterations ($p<0.001$). The most commonly observed anomaly was theincrease in the number of cusps in mandibular first molars.In our previous study on 30 T1DM patients[3], more than 50% showed characteristic dental anomalies and similar to this current study, the most commonly observed anomaly in those T1DM patients was six cusps in mandibular first molar.

Defect in the mineralisation process occurs in the dentition of these patients. Severe enamel hypoplasia observed in the 14 year old male patient is noteworthy. 5 T1DM patients showed root stumps, indicating rapid spread of caries in their less mineralised tooth structure.

1. Etiopathogenesis of Type I Diabetes Mellitus

Type 1 diabetes mellitus is autoimmune in etiology, where predominantly T cell mediated immune system is selectively destroying the beta cells of pancreas[1,2]. There are two important components in the etiopathogenesis, including genetic predisposition (30-40%) and environmental agents including viral infections, diet, gut microbiome, etc., acting on these genetically disadvantaged subjects.

Considering the immunogenetic component, more than 20 loci are associated with type 1 diabetes mellitus, the most important ones being IDDM1 and 1DDM2. IDDM1 lies in the major histocompatibility complex region on chromosome 6p21, which is involved in encoding several proteins responsible for immune response. DQB1 gene is of particular importance, as it encodes DQB1 peptide chain, which forms a part of the cleft in the surface of HLA class II molecule, which is essential for presenting peptide fragments of antigen to the T- helper lymphocyte.[8,9]

These HLA molecules present antigen to the T cells and the variation in the HLA genes can affect which antigens are presented and how effectively T cell recognise them.[8,9]

Genes like SL30A8 can influence the function and survival of insulin producing beta cells[8,9]

Added to these genetic factors, environmental factors like viral infections, diet and gut microbiome interact with these genetic predisposition and can trigger or accelerate the autoimmune process in T1DM.[8,9]

The systemic symptoms of T1DM include tiredness, malaise, lack of energy, muscle weakness, causing complications like blurred vision, and organ damage including renal damage, skin infections and

urinary tract infections. Diabetic ketoacidosis presenting with severe hyperglycemic conditions including nausea, vomiting, acidotic breath (Kussamaul breath), altered mood and clouding of consciousness are very common leading to coma, if left untreated[1,2,8,9].

In type 2 DM patients, the hyperglycemia develops slowly and the disease progresses several years before diagnosis. But in T1DM, the symptoms are very acute in duration to present before diagnosis[1,2,8,9]. Sudden weight loss and sudden polyuria within around one week before diagnosis, diabetic ketoacidosis leading to emergency admission were the most common signs and symptoms seen in our patients.

2. Systemic changes caused by diabetes mellitus

For studying the morphological alterations observed in the dentition of DM patients, three important systemic level molecular changes caused by DM are considered important, namely, 1) Hyperglycemia, 2) Chronic low grade inflammation in environment surrounding the odontogenic apparatus, and 3) Vascular changes and endothelial dysfunction in DM . The role of hyperglycemia and the role of chronic low grade inflammation, are discussed previously in our earlier articles.[3,4,5,6,7]Endothelial cells and their dysfunction plays a major role in most of the diabetic complications. These endothelial cells also play important role in changes observed in dentition, including enamel hypoplasia. Knowing the physiological functions of endothelial cells and mechanisms involved in endothelial dysfunction during diabetes mellitus can help us to understandbetter on what happens to the odontogenic apparatus during diabetes mellitus.

3. Blood vascular system and histology of endothelial cells

Blood vascular system forms the basis of human circulation by distributing oxygen, nutritive materials and hormones to the tissues and to collect carbondioxide and other products of tissue metabolism from the tissues, thereby sending them to the excretory system. Heart, pulmonary circulation (circulates blood to and fro from the lungs) and systemic circulation (circulates blood to and fro from all other tissues and organs of the body) forms the vascular system. Both these circulations are passed successively through large arteries, small arteries, arterioles, capillaries, venules, small veins and large veins and taken back to heart. The actual exchange of blood and the other nutrients, between the blood and the tissues take place in the minute thin walled capillaries and venules[10].

The endothelial cells form the main component of the capillary wall and is the living layer common to all parts of the vascular system including heart. These cells are elongated in the direction of the capillary axis with tapered ends, containing flattened oval nucleus with average diameter of about 8 microns. The endothelial cells are lined by a thin continuous basement membrane, which is again a product of endothelial cells. Pericytes surround the endothelial cells[10]. There are two main types of capillaries, namely continuous capillaries and fenestrated capillaries and also sinusoidal capillaries exist. The continuous or the muscular type, shows uninterrupted endothelium and is found in all parts, including muscles. The fenestrated type of capillaries has endothelium of varying thickness, and the thinnest area shows small pores closed by very thin membranous diaphragm. It is commonly seen in renal glomeruli, endocrine glands, intestinal villi, and elsewhere[10].

Capillary permeability is one of the most important histophysiological concerns, which is exchange of substances on both the ways across their walls. The capillaries have wide enormous surface, facilitating exchange and no significant transformation of energy is required for this process. Also endothelium acts like an inert porous membrane, which is permeable to water and crystalloids, and relatively impermeable to larger molecules. Endothelium is active in cell drinking (pinocytosis) as it contains numerous vesicles in submicroscopic form. These vesicles intake small quantities of fluid, transport it across the cell and discharge it into the perivascular space. In fenestrated capillaries, numerous small pores called fenestrae facilitate easy fluid movement faster than the continuous or muscular capillaries[10].

3a. Significance of endothelial cells

Vascular endothelial cells line the lumen of the blood vesselsand are basically exerting endocrine, autocrine and paracrine effects. Though these endothelial cells appear as an inert physical barrier, it is indispensable for maintaining vascular homeostasis under physiological conditions[11].

3b. Functions of endothelial cells in physiological conditions

Endothelial cells are found to be very important in enormous physiological functions including regulation of vessel integrity, vascular growth and remodeling tissue growth and metabolism, immune responses including inflammation, cell adhesion, angiogenesis, hemostasis and vascular permeability. The resting endothelium maintains themoment to moment balance between vasoconstriction and vasodilatation, prothrombosis and antithrombosis, pro-inflammation and anti-inflammation, pro-oxidation and anti-oxidation, and vascular smooth muscle cell growth promotion and growth inhibition. Thus it functions like a gatekeeper, thereby regulatingthe vascular tone, controlling inflammatory responses and tissue blood flow and also maintaining blood fluidity.Disturbing this tightly regulated equilibrium leads to endothelial dysfunction.

The vascular endothelium also acts like an endocrine organ, as it secrets a variety of vasoactive agents including prostaglandin(prostacyclin) I2 (PG12), endothelium-derived hyperpolarizing factor (EDHF), and nitric oxide (NO), and vasoconstrictors like angiotensin II, thromboxane A2, endothelin-1 (ET-1), and reactive oxygen species (ROS).Nitric Oxide and PGI2 inhibits platelet aggregation, whereas Von Willebrand factor produced by endothelial cells promotes platelet aggregation. Plasminogen activator inhibitor-1 (PAI-1) inhibits fibrinolysis[11].

3c. Nitric Oxide (NO)

Nitric oxide is produced within the endothelial cells and is very essential for maintaining vascular homeostasis. During the conversion of, L-arginine to L-citrulline,Endothelial nitric oxide synthase (eNOS) releases nitric oxide[12]. The nitric oxide released so, diffuses into the vascular smooth muscle cells (VSMCs) and activates soluble guanylatecyclase (sGC), causing increased levels of cyclic guanosine - 3,5 monophosphate and relaxation of VSMCs. Nitric oxide is also essential for preventing leukocyte adhesion and migration, smooth muscle cell proliferation and platelet adhesion and aggregation, thereby causing atheroprotective (antiatherogenic) effect along with opposing apoptosis and inflammation.

Nitric oxide have a half-life of less than 4 seconds[13]. It is rapidly metabolised to nitrite and then nitrate and get excreted through urine. NO is released by endothelial cells in response to shear stress exerted by the circulating blood or by substances such as Serotonin, Bradykinin, or Acetylcholine. NO is also an endocrine vasoregulator, that modulates the blood flow in the microcirculation[11,12,13,14,15,16].

4. Mechanisms causing endothelial dysfunction in DM

Endothelial dysfunction arises when there is reduced eNOS expression or reduced NO bioavailability.

4a. Reduced eNOS expression

Insulin plays a vital role in modulating eNOS activity. Insulin helps in achieving metabolic homeostasis by causing vasodilatation through eNOS activation[13,14,15,16]. During insulin resistance, insulin stimulated modulation of eNOS activation is hampered and thereby vasodilatation is prevented. From the other end, eNOS plays a major role in regulating insulin sensitivity especially in peripheral tissues. Experimental studies in mice lacking eNOS have shown to develop insulin resistance, thereby indicating that eNOS phosphorylation may be a novel target for the treatment of insulin resistance[13,14,15,16].

4b. Reduced NO bioavailability

Rapid degradation of NO reduces the bioavailability of NO. In diabetes mellitus, NO bioavailability is reduced due to increase in free oxygen radical ($O2 \bullet -$) levels in blood vessels. $O2 \bullet -$ reacts readily with NO and forms peroxy nitrite (ONOO-), thereby reducing NO bioavailability and preventing vasodilatation[13,14,15,16].

4c. Prostacyclin

Prostacyclin I2 is produced by the endothelium as a major metabolic product of arachidonic acid. After formation, PGI2 activates adenylatecyclase, which leads to the increase in production of cyclic AMP, and VSMC causing vasodilatation. PGI2 is also a proliferative agent of vascular smooth muscle cells and it reduces the oxidative stress and it prevents the adhesion of cells like platelet to vascular wall. In diabetes mellitus, there is a decreased secretion of PGI2 as shown in previous studies[11].

4d. Endothelium derived hyperpolarizing factor (EDHF)

NO mediates vasodilatation in relatively larger arteries and arterioles like aorta, whereas in smaller arteries Endothelium derived hyperpolarising factor predominantly affects endothelium- mediated vasodilatation. Thus, in larger arteries like aorta, reduced NO bioavailability marks the endothelial dysfunction, whereas in resistance arteries, NO, prostacyclins and EDHF are involved. Alterations in EDHF mediated responses are reported in diabetes mellitus[11].

5. Pathophysiological association between diabetes mellitus and endothelial dysfunction[4]

5a. Inflammation[16,17,18,19]

Metaflammation (Chronic low grade inflammation) is a prime factor in the development of diabetes mellitus. Hyperglycemia induced by diabetes mellitus can activate various proinflammatory factors and molecular pathways causing endothelial dysfunction. Nuclear Factor kappa-B pathway[19] (NF-kB) pathway is triggered by hyperglycemia causing production of cytokines and chemokines responsible for promoting inflammation and causing beta cell damage. Also along with NF-kB, Tumour necrosis factor–alpha (TNF–α), Interleukin -6 (IL-6) exerts pro-inflammatory effect on endothelial cells in diabetes mellitus[17,18,19].

5b. Oxidative stress[16,20,21]

Oxidative stress indicates imbalance between intra-cellular pro-oxidation and anti-oxidant system. In diabetes mellitus, intracellular hyperglycemia cause mitochondrial Reactive Oxygen Species (mtROS) generation, which in turn cause endothelial dysfunction. Low antioxidant activity or high levels of ROS causes mitochondrial dysfunction in endothelial cells. Mitochondria, being the power house of the cell, mitochondrial metabolism is responsible for the motility of endothelial cells, angiogenesis, proliferation and cell death. Thus, when hyperglycemia induces mitochondrial dysfunction, all the functions of endothelial cells are hampered, causing endothelial dysfunction[16,20,21].

Endothelial dysfunction starts earlier in the course of T1DM, because of accumulation of Advanced Glycation Endproducts (AGEs), caused by oxidative stress.

5c. Endothelilal to mesenchymal transition[16,22]

Endothelial to mesenchymal transition is a process where the endothelial cells get transformed into mesenchymal stem cells during certain pathological challenges to the body such as high oxidative stress, hypoxia, disturbed metabolism or shear stress force. In majority of the diabetic complications, endothelial to mesenchymal transitions is observed. When a proportion of endothelial cells undergo

transition, this may lead to endothelial layer disruption, forming plaque erosion, resulting in atherosclerosis.

5d. Cell death[23,24,25,26]:

Endothelial cells are prevented from cell death by the protective mechanism called autophagy. Autophagy helps in preventing cell death as it selectively engulfs the targeted substances, for instance mitophagy, lysophagy, Endoplasmic Reticulum (ER) phagy, etc., Autophagy helps in maintaining the endothelial function and is a life sustaining process.

Various pathophysiological conditions like tissue homeostasis, inflammation, immunology and cell growth invites cell death, which may be pre-programmed (apoptosis, ferroptosis, pyroptosis, necroptosis, etc.,) or accidental in occurrence (eg:necrosis). High glucose levels in DM prevent autophagy and induces apoptosis in endothelial cells. Thus, the protective effect of autophagy is prevented and various forms of programmed cell death including ferroptosis[25,26], pyroptosis is accelerated causing endothelial cell death in various organs during hyperglycemia. Apoptosis occurs in diabetic endothelial injury causing vascular injury and is induced by various factors like oxidative stress in the blood, disturbed blood flow, Advanced Glycation Endproducts (AGEs) and high levels of oxidised-Low Density Lipoprotein (ox-LDL)[16,23,24,25,26].

6. Endothelial dysfunction

"Endothelial dysfunction refers to the inability of endothelial cells to maintain vascular homeostasis due to the disturbed balance between endothelium-derived antiatherosclerotic factors and preatherosclerotic factors in favour of proatherosclerotic factors, leading to the initiation and progression of atherosclerosis"[11,16]. As discussed above, NO released from the endothelium has a variety of antiatherosclerotic effects such as vasodilation, inhibition of the proliferation of vascular smooth muscle cells, inhibition of leukocyte adhesion, and inhibition of platelet adhesion and aggregation. Therefore, endothelial dysfunction often refers to a condition in which increased NO inactivation and/or decreased NOproduction from the endothelium results in reduced NO bioavailability[11,12,13].

Diabetic patients are at high risk for developing microvascular and macrovascular complications. Diabetic vascular complications are major causes of morbidity and mortality in diabetic patients. Microvascular complications such as neuropathy, nephropathy, and retinopathy are major causes of decreased quality of life[16]. Macrovascular complications including coronary heart disease, cerebrovascular disease, and peripheral artery disease, are major causes of mortality[16]. Therefore, inhibiting the progression of atherosclerosis and preventing the development of diabetic vascular complications is clinically important for a better prognosis in diabetic patients[16]. In our study, diabetic complications were seen in around 5% of the T1DM subjects.

Diabetes mellitus is associated with endothelial dysfunction. Therefore, it is important to understand the mechanisms underlying endothelial dysfunction caused by diabetes mellitus and to select treatments that improve or augment endothelial function for preventing diabetic vascular complications[16].

7. Endothelial cells and odontogenesis:

7a. Instructive role of vasculature in odontogenesis:

Vasculogenesis and angiogenesis plays an important role in any organogenesis. Cranial vasculature beyond being involved in bringing nutrients, oxygen and removal of waste, also more importantly controls neural crest decisions[27,28,29]. Loss of endothelial cells cause changes in the extracellular matrix, which inturn impacts the neural crest cell survival and migration and can even lead to the cell death of cranial neural crest cells[27,28,29]. There are proven evidences for endothelial-neural crest co-ordination during early stages of cranial morphogenesis. Thus, vasculature is not only employed as transport

system, but also has an instructive role in controlling cell migration and in building organ architecture, thereby controlling formation of cranial structures like salivary glands, tooth, jaw, cartilage and bone[27,28,29].

7b. Endothelial cells and amelogenesis:

Ameloblasts, the enamel forming cells are highly differentiated cells, which are metabolically active and require a constant supply of oxygen and nutrients. Angiogenesis provides the necessary blood supply to the ameloblasts through dental follicle and surrounding tissues after the beginning of hard tissue formation[30].

Dental follicle development:

The dental follicle, a connective tissue sac surrounding the developing tooth germ, plays a vital role in guiding tooth eruption and maturation. Angiogenesis within the dental follicle ensures proper development and function of this structure, which in turn supports the ameloblasts[30].

Nutrient and oxygen delivery:

The newly formed blood vessels deliver oxygen and essential nutrients like calcium to the ameloblasts, allowing them to carry out their functions in enamel formation[30].

Maintaining pH and waste removal:

Angiogenesis facilitates the removal of waste products and metabolic byproducts from the developing tooth and surrounding tissues, contributing to a healthy environment for ameloblasts[30].

Influence on enamel maturation:

Proper blood supply from angiogenesis can influence the maturation and mineralization of the enamel, ensuring its strength and quality[30]. Also, blood vesselshelp in maintaining pH at formative zone during maturation process[30].

7c. Endothelial cells and dentinogenesis:

Instructive role of blood vasculature in odontogenesis[31]

Angiogenic- Odontogenic coupling is noted in the developing tooth bud regions, as Vascular Endothelial Growth Factor (VEGF) is highly expressed by the developing tooth buds. This is proved by the high expression of VEGF 1 and VEGF 2 postnatally by the endothelial cells located at the periphery of the tooth germ. Also, these endothelial cells show high immunoreactivity for VEGFR2[31,32,33,34]. These VEGFR positive capillaries lack smooth muscle coverage, expand and perforate the basal layer of odontoblasts before active dentinogenesis begins[31,32,34].

Reduced width of odontoblasts and dentin, delayed odontoblast maturation was observed when VEGFR2 was deleted from endothelial cells. Also, when VEGFR2 was deleted, the vasculature near odontoblast layer was lost by apoptosis. In VEGFR2 null mice, a decline in the phosphate pathway leading to defective mineralisation was noted[31,32,33].

These resultshighlighttheimportanceofthevasculatureinfacilitating post-natal tooth development and maintaining dentin mineralizationbytheprovisionofmultipleangiocrinefactors[34]

Angiogenesis supporting cell proliferation[30]:

Endothelial cells, through the formation of new blood vessels, ensure a constant supply of nutrients and oxygen to the developing tooth bud, supporting cell proliferation and differentiation[30,31,32].

Signaling molecules:

Endothelial cells secrete various signaling molecules, such as TGFβ1, Ptn, and Jag2, which promote odontoblast maturation and dentin formation[33].

Perivascular niches:

Endothelial cells can create perivascular niches, which are microenvironments surrounding blood vessels, that support the self-renewal and maintenance of dental pulp stem cells[30,31,32,33].

IL-6 signaling:

Endothelial cell-derived IL-6 (Interleukin-6) can activate STAT3 signaling in dental pulp stem cells, enhancing their self-renewal and promoting the formation of new blood vessels[33].

Vasculature as a Delivery System:

The vasculature, composed of endothelial cells and blood vessels, acts as a delivery system for essential nutrients and growth factors, while also removing waste products.

7d. Role of Endothelial Cells in Tooth Mineralization:

The interaction between endothelial cells and odontoblasts is crucial for tooth mineralization, with endothelial cells influencing odontoblast maturation and odontoblasts regulating capillary sprouting and plasticity[34].

Phosphate Delivery[34]:

Endothelial cells, deliver phosphate, a crucial component for dentin mineralization, to the odontoblasts.

Angiocrine Factors[34]:

Angiocrine factors are signalling molecules, primary growth factors and cytokines, released by endothelial cells. Endothelial cells release angiocrine factors, influencing their behavior and contributing to the mineralization process.

7e. Effect of endothelial dysfunction on odontogenesis:

As studied above, effective vasculature and healthy endothelium supports enamel and dentin formation in multiple ways, directly or indirectly, including their mineralisation process. During endothelial dysfunction, caused by DM, all the supportive effects of endothelium on odontogenesis is compromised. Ameloblasts may undergo cell death, because of the endothelial dysfunction or reduction in their number, leading to enamel hypoplasia. Also, endothelial dysfunction can cause disrupted phosphate pathway and defect in secretion of angiocrine factors,leading to defective mineralisation in tooth (both enamel and dentin) (Figure 5).

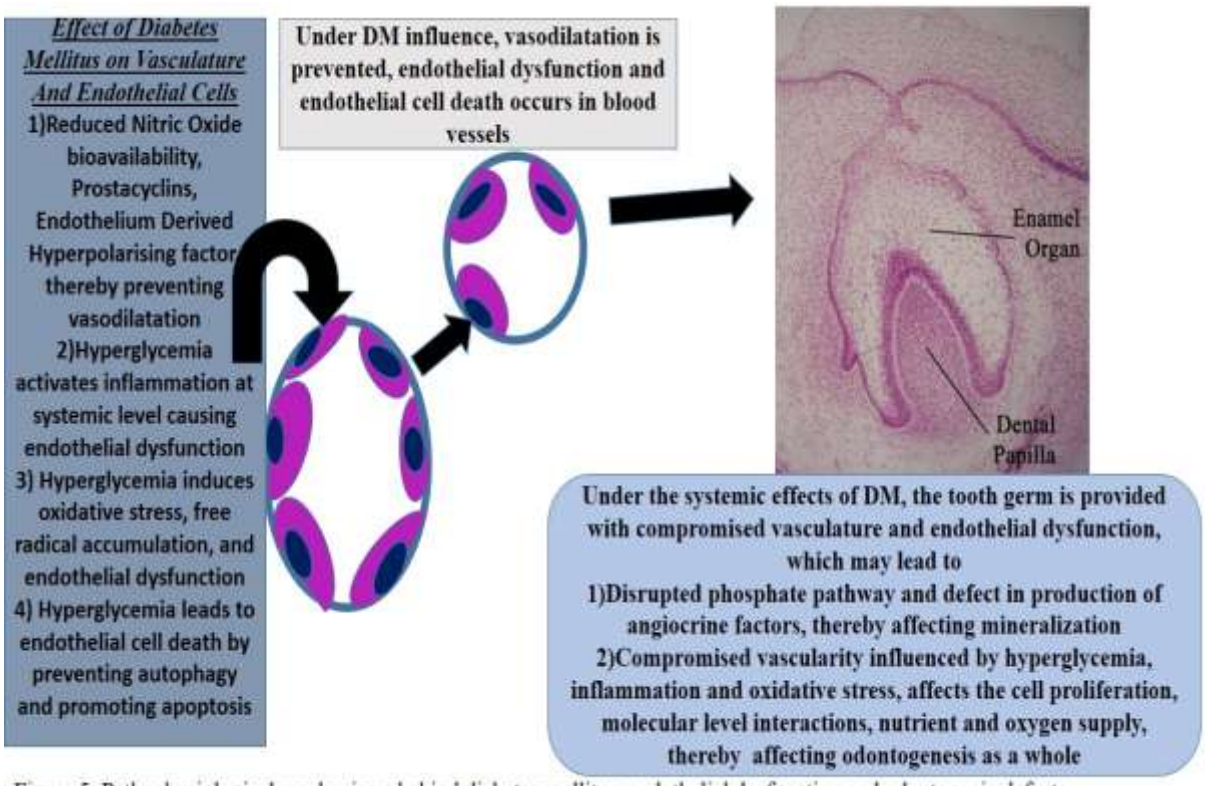

Figure 5: Pathophysiological mechanisms behind diabetes mellitus, endothelial dysfunction and odontogenic defects

8. Factors influencing odontogenesis and dental anomalies formation

8a. Odontogenesis – a complex process:

Odontogenesis is a complex process, which involves interaction between epithelium and ectomesenchyme, under the influence of genes, epigenetics and environmental factors, resulting in the formation and development of tooth[30]. The final phenotype of teeth is determined by every interaction taking place between epithelium and ectomesenchyme, which is directed by signalling molecules, protein receptors, and transcription factors acting at intracellular and extracellular level during various stages of tooth development. In oral cavity, each tooth will be on different developmental stages, at the same time period. Also, if two tooth germs are in the same developmental stage, they can be in different phase of growth, in that time. Thus, interactions of these multiple factors and their outcomes on dental phenotype during development are complex.Tooth itself can act as a record of many systemic pathologies, genetic changes, environmental insults that the body suffered during the process of tooth formation. Unlike bone, the changes impregnated on tooth enamel and dentin are permanent, as enamel and dentin do not undergo remodelling.

8b. Dental anomaly formation – a complex process too

The complexities observed in tooth formation are applicable even to a dental anomaly formation. The complexity and etiology is multiple in terms of factors, levels, dimensions, and progress in a specific period[35,36].

When a major environmental insult or a specific gene mutation is identified in relation to a dental anomaly (eg: fluorosis), on detailed investigation of the phenotype, it is common to have variation in phenotypic presentation between affected individuals (even if they are from the same family), variation between primary and permanent dentitions of the same individual, and between different teeth of the permanent dentition (or deciduous dentition) in the same person[35,36].

It is also important to understand that spatial time during which the environmental insult or genetic mutation occurs is also a major component in determining the phenotype of dental anomalies. Considering the genetic influence, if mutations and deletions occurs in two different time periods in the same genes (eg: AMELX and DSPP genes), it can result in two different phenotypes. Thus, phenotypic variation in dentition is dependent not only on the severity of genetic defect, but also on the time of occurrence and site of the change and its effect on the protein product deposited over the forming hard tissue. It is also important to understand that closely similar dental anomalies (eg: any dental anomaly in relation to size or shape, etc.,) can have different etiologies; and notoriously, similar to genetic mutations, environmental insults can result in similar phenotypic anomalies also.

When genetic mutation occurs in genes active in odontogenesis, those developmental regulatory genes can have action on multiple tissues and organs of the body, thereby resulting in syndromes with dental anomalies as a part of the syndrome (eg dental anomaly in Rubenstein Taybe syndrome). On the other hand, the causation is different when the developmental dental anomaly is secondary to the systemic effects of the syndrome (eg: enamel defects seen in Coeliac disease or vitamin D resistant rickets)

Thus, with this broad understanding of the multifactorial nature of the dental anomalies, in addition to looking for the mutation of single genes as the causative factor of dental anomaly, we should also explore the complex backround. When there is intricate balance between regulatory genes, and their activators or inhibitors, normal phenotype of teeth in relation to their size, shape and number occurs. Odontogenesis is a well programmed and sustained process that, any delay in balance between forming cells and signals, upto 24 hours is delicately balanced by substitution or recovery, and normal morphology is achieved. Anomalies like hypodontia or supernumerary teeth occur if the positive feedback loop between genes in the dental ectomesenchyme is disturbed. Toothanomalies occur if the normal balanced control of Shh or Wnt is upregulated or downregulated by any environmental or epigenetic factors. Thus, molecular and cellular interactions, both at intracellular and extracellular level determine the final macroscopic phenotype. These interactions are based on three important factors, namely genetic, epigenetic and environmental control[35,36].

8c. Diabetes mellitus and dental anomalies

From our study results, it is shown that diabetes mellitus affects the odontogenic apparatus. Our study results show that dental anomalies are more common in T1DM patients (61%), compared to the normal population (12.9%). Also, in our previous study, around 50% of the T1DM patients have shown dental anomalies. Prevalence of sixth cusp in mandibular molars (Entoconulid), tuberculumintermedium or tuberculumsextum in normal population ranges from 6.4 %, whereas in T1DM population, it was found to be 29%. Thus, association of dental anomalies with T1DM is well established.

The etiological factors may be multidimensional including genetic, epigenetic and environmental changes caused by diabetes mellitus. The most common environmental changes caused by diabetes mellitus includes hyperglycemia, chronic inflammation and vascular changes. The epigenetic changes in diabetes mellitus, influence of hyperglycemia on tooth formation and the role of inflammation in tooth anomalies formation are discussed by us in detail in our previous articles. The role of vascular changes and endothelial dysfunction in DM and its effect on odontogenesis is discussed in detail in this article. Endothelial dysfunction can cause enamel hypoplasia directly and dental anomalies indirectly.

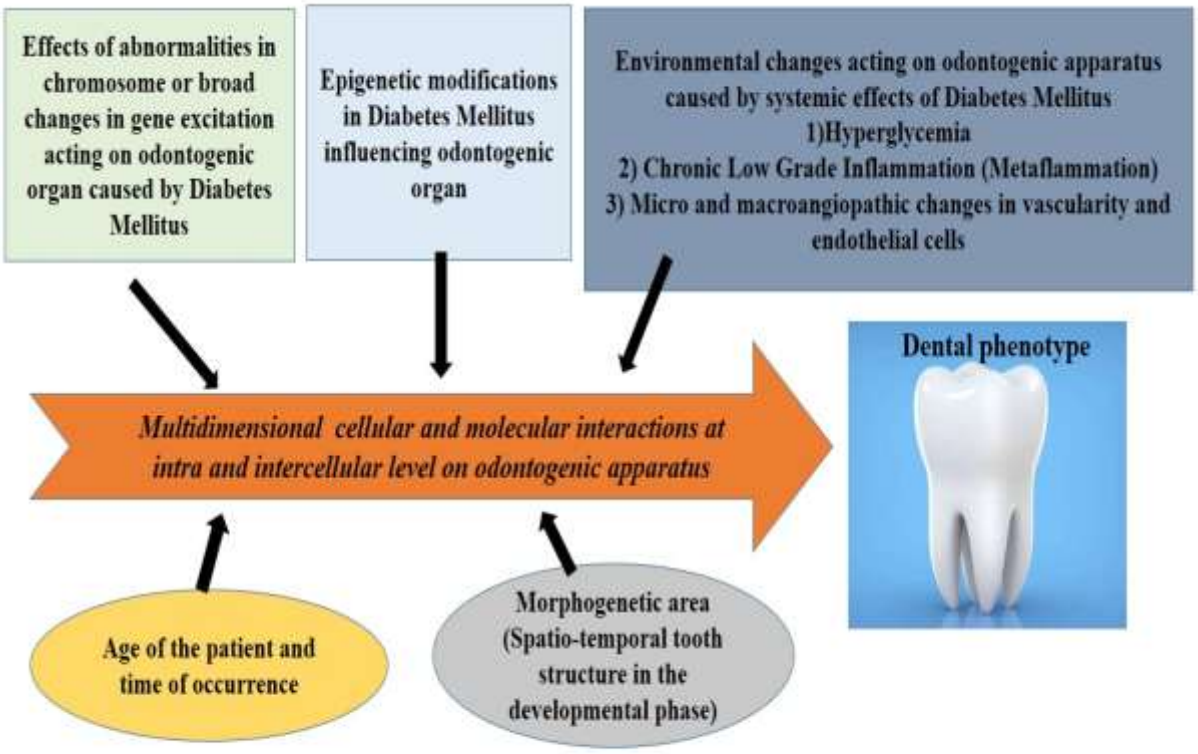

Figure 6: Factors affecting odontogenesis in Type 1 Diabetes Mellitus patients

CONCLUSIONS:

T1DM show statistically significant ($p<0.001$) increase in the prevalence of dental anomalies when compared to control subjects, and the most common dental anomaly observed are presence of sixth cusp in mandibular molars, followed by more number of prominent grooves and pits on the occlusal surface of maxillary and mandibular molars and premolars, and enamel hypoplasia. Thus, diabetes mellitus have systemic effect on all the organs of the body, including tooth. In T1DM, the systemic changes caused by DM affect the tooth formation significantly, as odontogenesis also occurs in the same younger age group. Multiple etiological factors including genetic, epigenetic and environmental factors are involved in this complex epithelial ectomesenchymal interactions occurring during dental anomalies formation in DM. We aimed at explaining the endothelial dysfunction occurring in DM and its potential role in occurrence of dental anomalies. A solid understanding of this long and fascinating odontogenic process, both in disease and health, becomes the need of the hour, as it carries the information on the systemic changes occurring in the early days of life, without undergoing remodelling. Also, these changes can be a potential indicator for early diagnosis of T1DM. Further studies exploring the genetic changes in these T1DM patients can shed more light on this topic.

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