Review Article

Molecular Insight into Odontogenesis in Hyperglycemic Environment: A Systematic Review

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Abstract

Diabetes mellitus is an endocrinal disorder affecting worldwide and the disease incidence is rising alarmingly high. The effects of diabetes on tooth development are explored by limited studies and their molecular insights are very rarely studied. This systematic review is aimed to provide the best scientific literature source on the molecular insights into odontogenesis in hyperglycemic environment caused by diabetes mellitus or by maternal diabetes on the offspring. The literature search was conducted on the databases, namely PubMed, PubMed Central, Cochrane, and Scopus. The original studies exploring the alterations in the molecular pathways of odontogenesis in diabetes mellitus were selected. Data were extracted, chosen, and evaluated by two independent researchers. At the end of thorough data search, four articles were eligible for the review. Three articles brought out the molecular pathways involved in the offspring of gestational diabetes through animal models. Fourth article was an *in vitro* study, which treated the stem cells in hyperglycemic environment and drafted the molecular pathway. The altered molecular pathways in dental epithelial stem cells (DESCs), dental papilla cells (DPCs), and stem cells from apical papilla were studied and empowered with statistical analysis. Thus with this systematic review, we conclude that apurinic/apyrimidinic endonuclease1 downregulation causing deoxyribonucleic acid hypermethylation and *Oct4, Nanog* gene silencing, activation of toll-like receptor-4/nuclear factor kappa B (TLR4/NF-κB) pathway are involved in suppressing cell proliferation and accelerated apoptosis in DESCs in high glucose environment. DPCs are suppressed from odonto differentiation by activation of TLR4 signaling and resulting inhibition of SMAD1/5/9 phosphorylation in diabetic condition. NF-κB pathway activation causes decreased cell proliferation and enhanced differentiation in apical papilla stem cells in hyperglycemia. Further studies targeting various stages of odontogenesis can reveal more molecular insight.

Keywords: *Diabetes mellitus, hyperglycemia, maternal diabetes, odontogenesis, tooth development*

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Introduction

"Diabetes mellitus (DM) refers to a group of common metabolic disorders that share the phenotype of hyperglycemia.[1] Depending on the etiology of the DM, factors contributing to hyperglycemia include reduced insulin secretion, decreased glucose utilization, and increased glucose

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production. It will be the leading cause of morbidity and mortality for the foreseeable future."[1] The resulting hyperglycemic condition in offspring due to gestational DM causes epigenetic signature on various organs, resulting in altered phenotypic expression, especially during organogenesis.[2]

Odontogenesis is a multifactorial process, where tooth germ derived from ectoderm and undifferentiated mesenchymal cells interact to produce enamel, dentin, pulp, cementum, and related structures.[3] Highly differentiated cells, such as ameloblasts, are more sensitive even to minor changes in the environment, and thus the tooth formation in toto can be affected by various congenital, metabolic, or developmental disorders and also by local environmental factors.[4]

The number of studies on the effect of DM on tooth development is very few. Dental caries,[5] periodontal problems,[6] enamel hypoplasia,[7] altered rate of tooth eruption,[8] microscopic changes[9] (such as defective enamel rods, decreased rate of mineral deposition in dentin, thickened basement membrane in pulp, increased pulp stones, decreased calcium, and phosphorous content),[10] increased magnesium content, morphological alterations[11] (such as increased number of cusps), and altered tooth size are reported in the literature as the effect of this metabolic disorder. However, the molecular insight into these changes is not well understood, as the studies at molecular level are countable.

Thus, our study was aimed to have a systematic review on the research focusing on the molecular pathways behind odontogenesis in hyperglycemic environment.

Materials and Methods

Data sources and search strategies

The article search was carried out by two prime investigators in the PubMed database using the search terminologies "diabetes mellitus and tooth development" and "diabetes mellitus and dental hard tissues." The search was also extended to the PubMed Central, Scopus, and Cochrane databases in a similar way, and Zotero software was used for saving the articles and acknowledging the duplicated ones. Efforts were made to include all available articles in the English literature, and the other language articles were excluded from the study. The articles were also searched by using the references quoted in the obtained articles. The abstracts of the articles without full text and the unpublished articles were excluded from the study, and the authors were not contacted for the full text.

Study selection

The original genetic studies focusing on the molecular pathways in odontogenesis in DM (hyperglycemic) condition or offspring of maternal diabetes are included in this review. Reviews, case series, and other language articles are excluded from this study. Any controversies in the inclusion and exclusion of articles are resolved after relevant discussions by the two researchers.

Data extraction

Information regarding the first author, publication year, country, type of study, sample size, and advanced research methodologies used to elicit the molecular pathways are tabulated in Table 1.

Synthesis of results and analysis

The results of these molecular studies are compiled after thorough analysis, and meta-analysis is not performed in this data summary. This systematic review provides

Table 1: Data extracted from the selected review articles

S. No.	Name of first author/year	Country	Type of study	Sample size	Techniques used in the methodology
1	Chen *et al*.,[12] 2016	China	Experimental study–animal model	54	Cell culture, RT-PCR, Western blot, and immunocytochemistry
2	Chen *et al*.,[2] 2017	China	Experimental study–animal model	54	Genomic DNA and total RNA isolation, microarray gene expression, primary DESCs culture, cell growth and colony formation assay, immunostaining, RT-PCR, Western blot, sodium bisulfate sequencing, global methylation analysis, and micro-CT
3	Lyu *et al*.,[13] 2020	China	Experimental study–animal model	54	Micro-CT, RT-PCR and Western blotting, immunostaining, cell culture and treatment, and small interfering RNA treatment
4	Wang *et al*.,[14] 2019	China	Experimental study–*in vitro*	6	Cell culture, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2,5-tetrazolium bromide assay, alkaline phosphatase activity, alizarin red staining, RT-PCR, and Western blotting

a narrative review and consistent summarization of the involved molecular pathways.

Results

In our search through the previously specified databases, a total of 5386 articles are identified and additionally five articles are included after going through the references cited in the obtained articles. By title reading, 124 articles are selected, thereby eliminating a total of 5267 irrelevant and duplicated articles. In these 124 articles, abstracts are read in 119 articles and 84 articles are excluded after reading the abstract. The remaining five articles with unavailable abstracts are also excluded. Thus, we arrived at a total of 35 eligible articles, of which, the original studies focusing on the macroscopic or microscopic dental alterations in DM or offspring of gestational diabetes; studies addressing the changes in clinical patterns of tooth eruption or changes in clinical parameters due to DM; and studies focusing on dental caries, periodontitis, and peri-implantitis in tooth due to DM, reviews and case series were excluded from this systematic review [Figure 1]. In the end of our thorough literature search, we finally arrived at four articles. Two of the selected articles were from the same authors, but addressing on different molecular pathways and were included in this review. The application of molecular research methodologies pertaining to DM and odontogenesis was initiated only in the recent years, and that too only by few researchers, and we chose those four recent molecular studies.

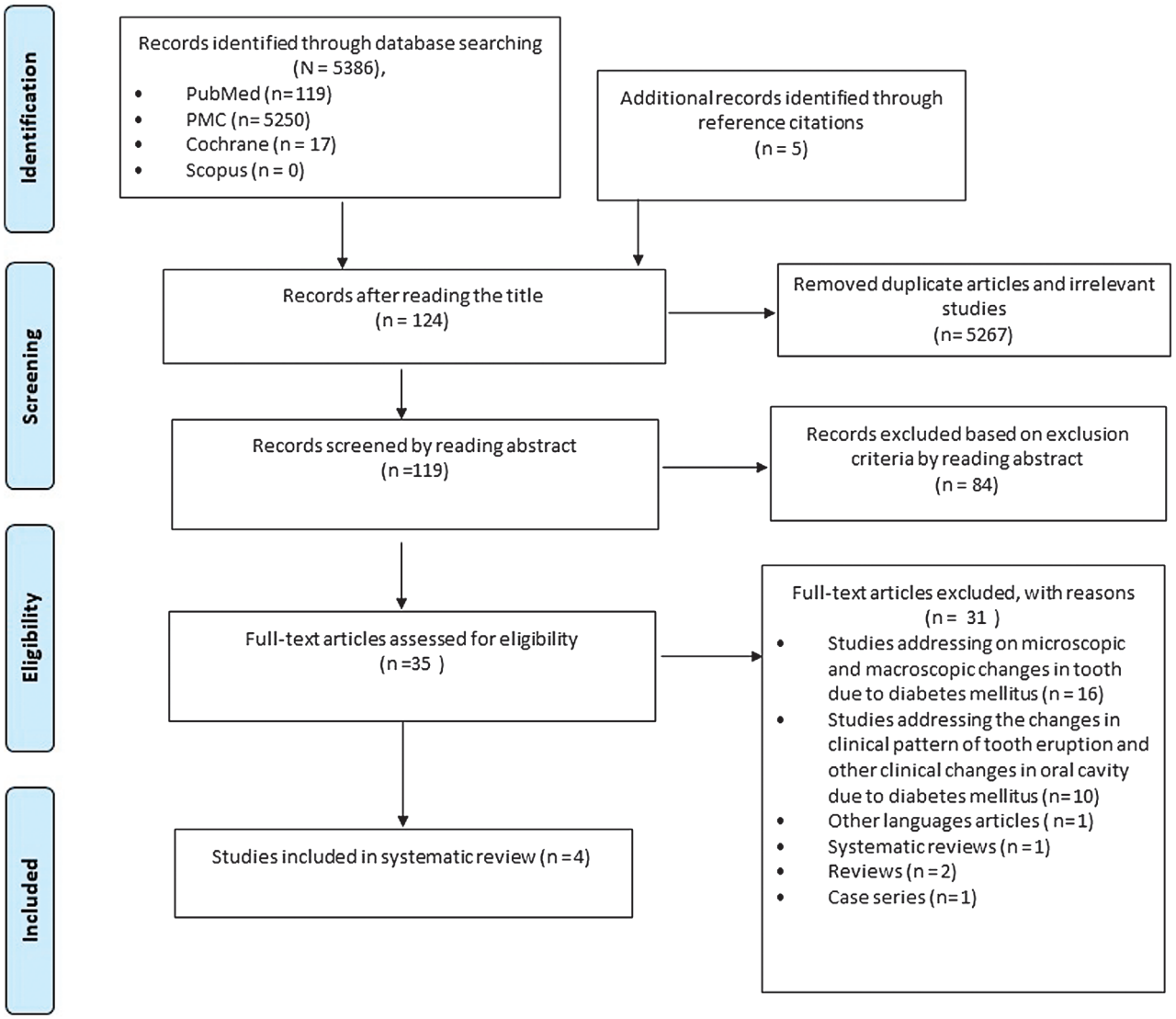

Figure 1: Flowchart showing selection of articles for systematic review (preferred reporting items for systematic reviews and meta-analyses [PRISMA])

Study characteristics

The authors of the selected four articles were from the same country, China, and three of these original articles were animal studies, and the remaining one was an *in vitro* study. In the animal studies (n = 3), the researchers established gestational DM (GDM) rat model by intraperitoneal injection of streptozotocin (STZ), and their offspring were studied for establishing the molecular pathways. In the *in vitro* study, diabetes-like hyperglycemic environment was created, and the apical papilla cells were isolated and studied to arrive at the molecular insight. These studies were published in the years between 2016 and 2020.

Altered molecular pathways explored during odontogenesis in hyperglycemic environment

In the first study by Chen *et al.*,[12] the pregnant maternal diabetes rats were divided into three groups, namely controls (n = 18), diabetic group (n = 18), and diabetic group with insulin treatment (n = 18). In the diabetic group and diabetic with insulin treatment group, diabetes was induced by the injection of STZ at a dosage of 75 mg/kg of body weight on E9.5 of pregnancy, when the odontogenesis begins. Their male offspring alone were included in the further research. At E15.5, E17.5, and postnatal 0.5 day, the mandibular specimens of the offspring were isolated and studied. The tooth germ of the first molar was studied for the detection of genetic expression through their dental papilla cells (DPCs) and dental epithelial stem cells (DESCs). Cell culture, real-time polymerase chain reaction (RT-PCR), Western blot, immunohistochemistry (IHC) were performed and cell proliferation and apoptosis detection, small interfering ribonucleic acid (RNA) treatment (siRNA) were added to the procedures. Mean ± standard deviation (SD) of each group was analyzed, and t tests or analysis of variance (ANOVA), followed by Bonferroni test were carried out to arrive at P value ($P < 0.05$ was considered statistically significant). The offspring from diabetic group presented with significant body weight loss and decreased Ki67 expression in the tooth germ and high number of TUNEL-positive cells, indicating decreased cell proliferation and enhanced apoptosis. Also, IHC and Western blot revealed increased expression of TLR4, MYD88, TRAF6, and p65, indicating the involvement of Toll-like receptor-4/nuclear factor kappa B (TLR4/NF-κB) pathway. Further, the levels of pro-inflammatory cytokines such as interleukin (IL)-1α, IL-1β, IL-6, and tumor necrosis factor (TNF)-α were also raised. All these effects were reversed after insulin treatment in the third group. Thus, the authors conclude that high glucose environment activates TLR4/NF-κB pathway in a dose-dependent manner, which leads to activation of pro-inflammatory cytokines, resulting in the decreased cell proliferation and increased apoptosis in DESCs of molar tooth germ and DPCs, resulting in odontodysplasia [Figure 2].

The same authors published their next research in 2017,[2] where they explored how GDM suppressed the incisor enamel formation and DESCs proliferation

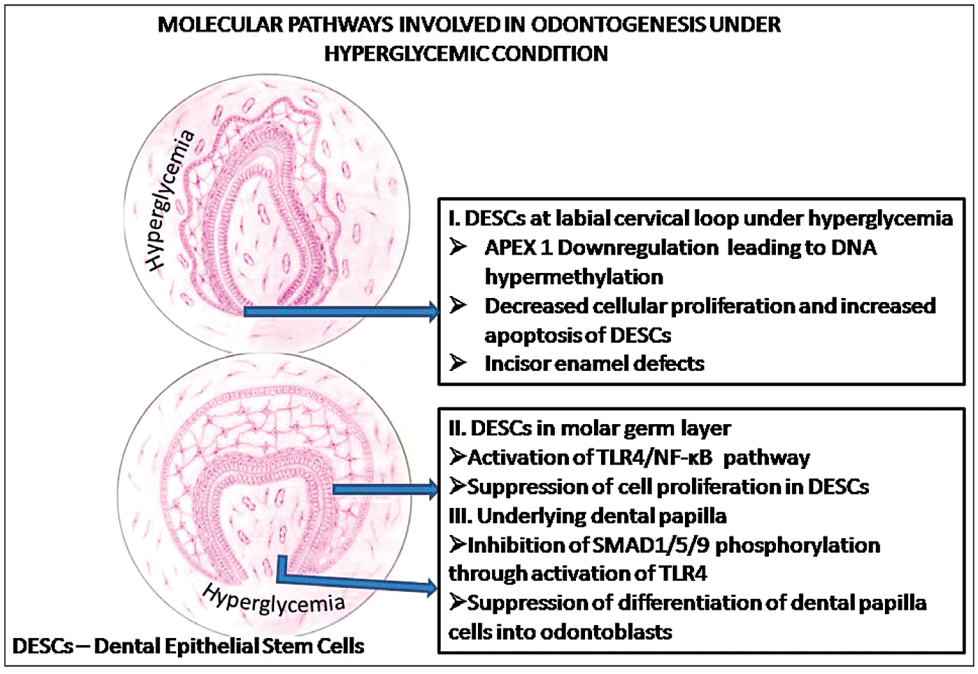

Figure 2: Molecular pathways elicited in the tooth development in hyperglycemic environment

in the offspring through epigenetic gene silencing. In a similar animal model as described in the previous article,[1] they harvested the DESCs from the labial cervical loop (LaCL) and explored the involvement of apurinic/apyrimidinic endonuclease1 (*APEX*1) downregulation by genomic deoxyribonucleic acid (DNA), total RNA isolation, microarray gene expression, DESCs culture, cell growth, and colony formation assays, and *APEX*1 knockdown. IHC, RT-PCR, Western blot, sodium bisulfate sequencing, global DNA methylation analysis, and micro-computed tomography (CT) were also used. Here, the authors conclude that GDM lays its epigenetic signature by causing downregulation of *APEX*1. *APEX*1 is a mammalian protein essential for maintenance of cell survival and maintenance of stem cell pool through its redox homeostasis function. Downregulation of *APEX*1 causes DNA hypermethylation in the Oct4 and Nanog promoter regions, by increased production of DNA methyltransferase enzyme 1 (DNMT 1), which results in gene silencing in *Oct4* and *Nanog*. Thus, *Oct4* and *Nanog* genes, which are known to regulate stem cell proliferation and self-renewal, is significantly downregulated in the offspring of diabetic dams. Thus, cell proliferation in DESCs of LaCL is significantly suppressed, resulting in dental deformities. High glucose induces DNMT1 upregulation by *APEX*1 inhibition or by upregulating extracellular signal-regulated kinase (ERK) and Jun amino-terminal kinase (JNK) signal activation [Figures 2 and 3]. These ERK and JNK pathways are involved in the transcription of DNMT1 expression. Thus, the authors conclude that DM is directly linked to cause epigenetic silencing of genes, such as *Oct4* and *Nanog*, through DNA hypermethylation, and *APEX*1 downregulation is a key factor involved in this mechanism.

The third article by Lyu *et al.*[13] analyzed the effects of GDM on odontoblastic differentiation by studying the DPCs. DPCs are differentiated into odontoblasts, and the remaining undifferentiated DPCs form the dental pulp in the future. The hyperglycemic environment produced by GDM in the offspring affects the odontogenic differentiation of DPCs by activation of

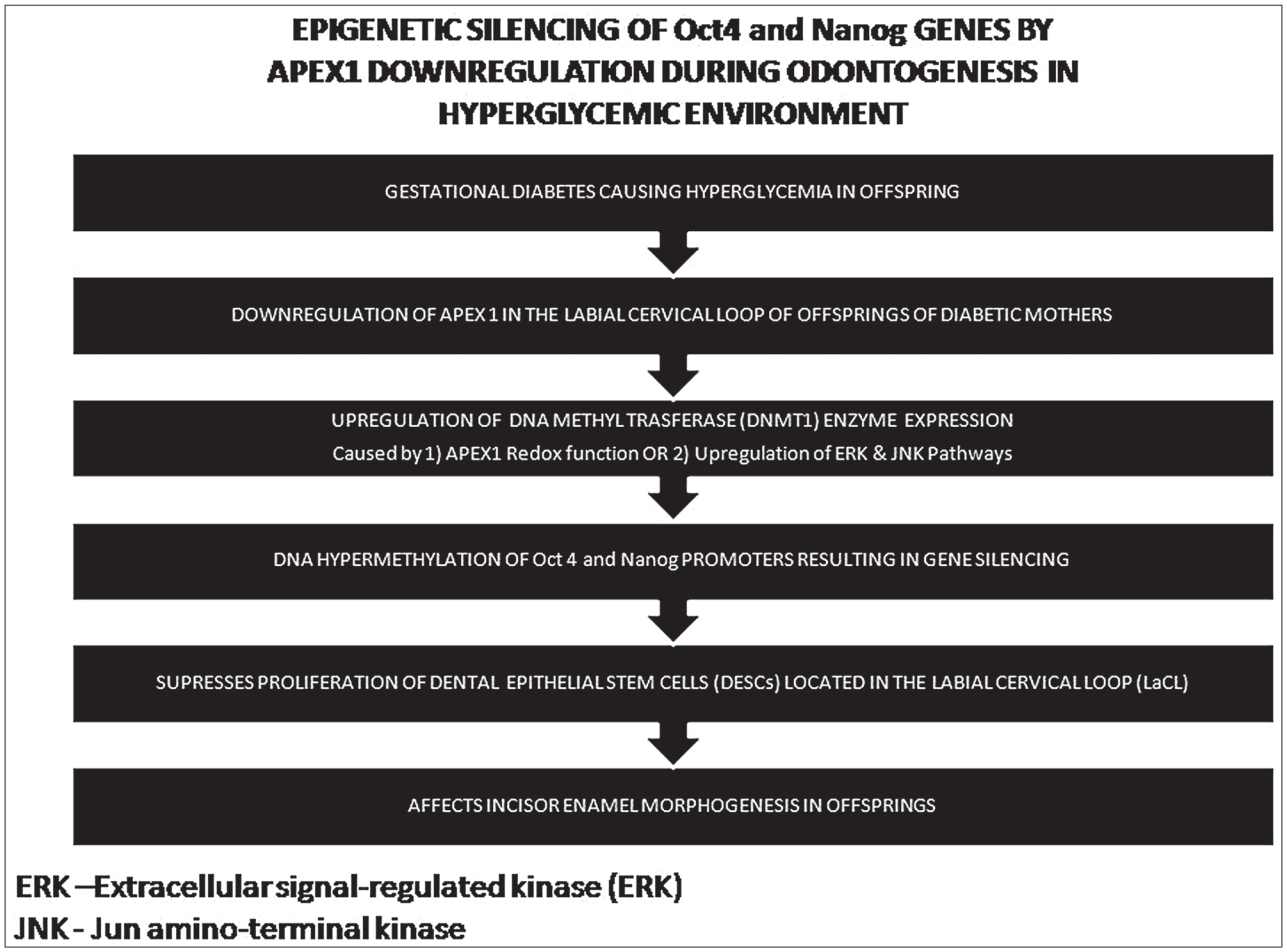

Figure 3: Flowchart showing deoxyribonucleic acid hypermethylation of dental epithelial stem cells through apurinic/apyrimidinic endonuclease 1 downregulation

TLR4/NF-κB p65 pathway in molar germ. TLR4 is an important molecule involved in cell proliferation, apoptosis, and mineralization on tooth germ during odontogenesis.[1] The authors of this article concluded that activation of TLR4/NF-κB p65 pathway by high glucose environment causes inhibition of SMAD1/5/9 phosphorylation in DPCs. This BMP/SMAD signaling is essential for the conversion of DPCs to odontoblasts, and here the study results show that maternal diabetes causes suppression of odonto differentiation by the suppression of SMAD1/5/9 phosphorylation [Figure2].

The fourth article considered in this review by Wang *et al.*[14] is an *in vitro* study where the stem cells from the apical papilla (SCAPs) were isolated from the immature dental roots and cultured. MTT assay abbreviated as 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2,5-tetrazoliumbromide, alkaline phosphatase activity monitoring, alizarin red staining, RT-PCR, and Western blot analysis were performed. The results showed that high glucose affects the odonto/osteogenic differentiation of SCAPs by the activation of NF-κB pathway. The high glucose is transported into the cytoplasm of the SCAPs by glucose transporters (GLUT). The p50/p65 complex in the cytoplasm is phosphorylated, which causes IκBα hydrolysis and translocation of p65 into nuclei from the cytoplasm. This causes the activation of NF-κB pathway and enhances the odonto/osteogenic differentiation of SCAPs [Figure 4]. The authors conclude that high glucose reduces the proliferation of SCAPs, but enhances the osteo/odonto differentiation of SCAPs.

Discussion

DM, being the most common disease worldwide, causes severe degenerative changes in the target organs. Also, hyperglycemia caused by gestational diabetes is considered as the most important "teratogen" to the developing fetus.[12] High mortality and morbidity rate and destructive organogenesis in the fetus due to maternal diabetes has alarmed the medical world. Tooth, being one of the important hard tissues of the body, also suffers from hyperglycemic environment, but the studies targeting toward this arena are limited. The studies[5-7] correlating dentition and DM speak a lot about dental caries, periodontal issues, and periodontitis. Earlier articles, with little advancement in research, speak about biphasic effects of diabetes on dental development as it causes accelerated tooth

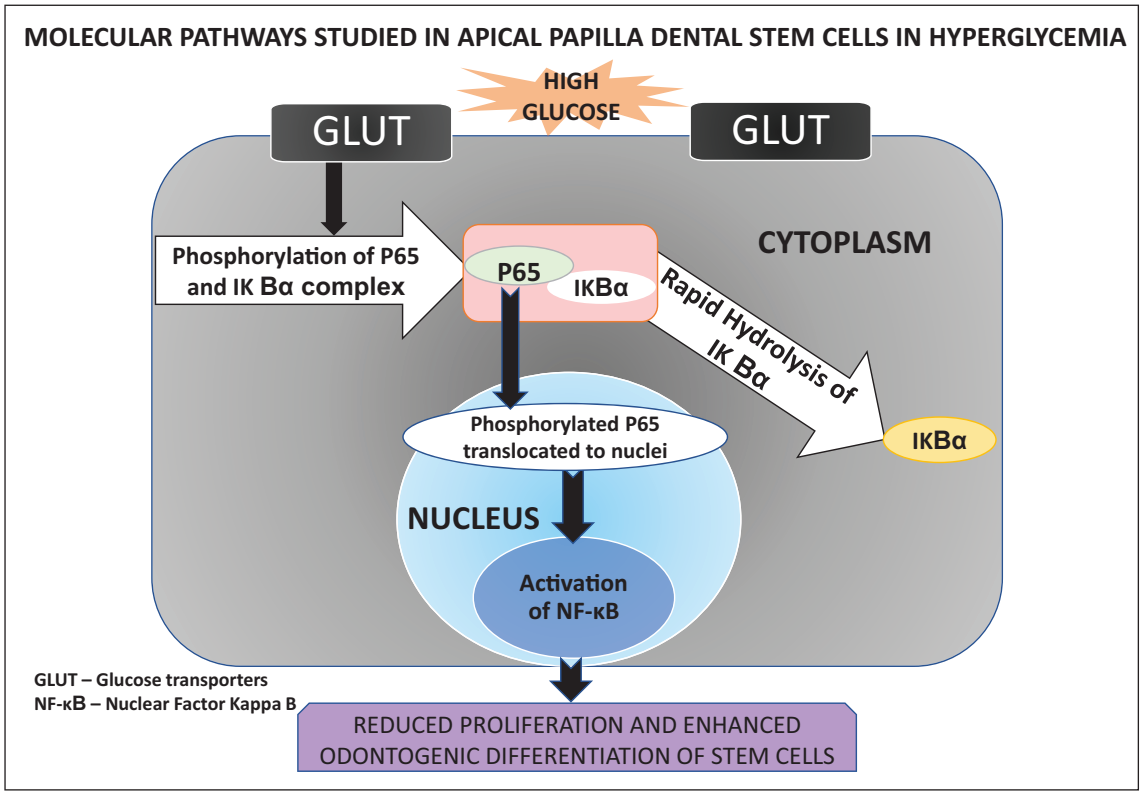

Figure 4: Activation of nuclear factor kappa B pathway by high glucose level

eruption before 11.5 years of age and delayed dental eruptions after 11.5 years.[15] Morphological changes, enamel hypoplasia, and microscopic alterations in the structures of enamel, dentin, and pulp were observed in diabetic conditions, and the researchers believed that diabetes definitely affects the formation and mineralization of tooth.[8-11] With advancements in the molecular research, we are able to prick into the molecular pathways involved in those changes, and to the best of our knowledge, our systematic review is the first to compile them.

DM affects both DESCs and the dental papilla stem cells during odontogenesis, thereby affecting enamel, dentin, pulp, and whole of periodontium. Their molecular pathways are brought into limelight by the four articles[2,12-14] taken for our review. Of the four studies, two[2,12] were by the same authors conducted on their animal model. The DESCs in molar germ and LaCL of incisors were studied, and it was concluded that the high glucose suppresses the cell proliferation and enhances the apoptosis of DESCs. These DESCs give rise to inner enamel epithelium, stratum intermedium, stellate reticulum, and outer enamel epithelium during odontogenesis, and the decreased proliferation of DESCs could be responsible for clinically observed enamel hypoplasia in patients with diabetes. DNA hypermethylation of *Oct4* and *Nanog* gene through *APEX*1 downexpression is reported as the molecular alteration behind the suppression of DESCs in incisor model. Activation of TLR4/NF-κB pathway in DPCs and DESCs is proved to be the molecular pathogenesis for the suppressed cell proliferation and enhanced apoptosis in molar tooth germ.

The DPCs are suppressed from differentiating into odontoblasts by the activation of TLR4 signaling pathway in molar tooth germ, which causes suppression of SMAD1/5/9 phosphorylation in the underlying dental papilla in diabetic condition.[13] Thus, it may affect the phenotypic expression of dentin and related structures. From these molecular insights, we can visualize how the altering levels of blood glucose can determine the resulting phenotypic expression of dental organ. Also, these adverse effects are reversed in the treated diabetic offspring in the aforementioned studies,[2,12,13] emphasizing on the need of treatment for this metabolic disorder, especially during the period of organogenesis.

Wang *et al.*[14] support the study results of Chen *et al.*[12] that NF-κB is activated by high glucose, causing decreased stem cell proliferation, but enhancing differentiation of these stem cells. Enhanced differentiation is a new insight provided by these authors, which needs further magnification in future days through advanced molecular studies.

Because of the limited studies and the same involved authors, the results could not be compared with other studies. The statistical analysis sounds reliable. Newer studies targeting the morphological stages of odontogenesis can reveal more molecular aspects.

Our systematic review has included all possible pathways elicited so far in the English literature, and the limitations include limited available research on the subject. Further studies in larger scales are needed to handle this metabolic disorder effectively.

Conclusion

Only few studies are conducted in the molecular aspects of odontogenesis in hyperglycemic environment. DM causes epigenetic signature by causing gene silencing in *Oct4* and *Nanog* genes through *APEX*1 downregulation and resulting in DNA hypermethylation in DESCs of incisor models. Hyperglycemia affects offspring of diabetic dams by suppression of cellular proliferation and enhanced apoptosis in molar tooth germ by activating TLR4/NF-κB pathway. Odontoblastic differentiation of dental papilla stem cells is suppressed by the activation of TLR4 signaling and resulting in the inhibition of SMAD1/5/9 phosphorylation in dental papilla. High glucose environment decreases apical papilla stem cell proliferation and enhances their odonto/osteogenic differentiation. Further studies in larger scales are needed to correlate these molecular findings clinically and for treatment purpose.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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