Original Article Impact of Metformin on Odontoblastic Differentiation and Root Formation During Tooth Development of Diabetic Rat's Offspring

Yasmine Adel Sabry, Asmaa Saleh El Mahdy, Fatma Ibrahim and Mahitabe Elgamily

Department of Oral Biology, Faculty of Dentistry, Mansoura University, Egypt

# ABSTRACT

**Introduction:** Gestational diabetes mellitus (GDM), a clinical class involving pregnant patients with diabetes during pregnancy, negatively impacts fetal development, leading to defects in dental enamel and deciduous teeth abnormalities.

Aim: Evaluating the effect of metformin on odontoblastic differentiation, root formation and tooth development in offspring rats born from diabetic mothers.

**Methodology:** After induction of pregnancy in 12 adult Sprague-Dawley female albino rats weighing 180-200g and confirmation of day zero of pregnancy, rats were subsequently divided into three groups (N=4 each). Group I (Non-diabetic control): the pregnant female rats were administered citrate buffer solution only. Experimental GDM was induced in groups II and III via injection of a single shot of streptozotocin (STZ) 45 mg/Kg body weight intraperitoneally. Group II was left untreated, Group III was treated with metformin 50mg/kg body weight once daily from the 4th day of pregnancy until birth. Thirty-six offspring rats were obtained (12 from each group). Six offspring from each group were sacrificed at 10 and 21 days after birth. The heads were collected, demineralized and processed, then stained with hematoxylin and eosin, and Nestin immunohistochemical staining. The results were evaluated quantitatively using image analysis followed by one-way ANOVA and post-hoc Tukey statistical analysis.

**Results:** Diabetes negatively affected the odontogenesis process. Ameloblasts and odontoblasts showed degenerative changes accompanied by delaying in the root formation and eruption. A significant decrease in Nestin expression was noticed in the diabetic group. Treatment with metformin improved the cellular state, and also significantly improved the root length, Nestin expression, and odontoblastic differentiation.

**Conclusion:** Treatment with metformin ameliorates the adverse effects of gestational diabetes on odontogenesis process, eruption state and odontoblastic differentiation in offspring rats born from diabetic mothers. So, it is recommended that females with gestational diabetes be treated with metformin to protect the tooth development of their offspring.

Received: 21 February 2024, Accepted: 12 March 2024

Key Words: Gestational diabetes mellitus; metformin; nestin; odontoblasts; tooth development.

**Corresponding Author:** Mahitabe Elgamily, Associate Professor, Department of Oral Biology, Faculty of Dentistry, Mansoura University, Egypt, **Tel.**: +20 10 0077 7402, **E-mail:** Mahitabe.fathy@yahoo.com **ISSN:** 1110-0559, Vol. 48, No. 1

#### **INTRODUCTION**

Gestational diabetes mellitus (GDM) is a frequent problem linked with pregnancy leading to unfavorable fetal outcomes<sup>[1]</sup>. The hyperglycemic state lead to altered phenotypic expression during organogenesis of the offspring<sup>[2]</sup>. Studies showed that GDM causes considerable bone loss and hypomineralization in teeth by affecting the functioning cells and the concentration of the inorganic minerals in the hydroxyapatite structure of dentin and enamel. Therefore, many of the mechanical characteristics in offspring's teeth may be adversely affected. Few studies declared its damaging effect on odontoblasts and dentin formation<sup>[3]</sup>. The principal treatment strategy for GDM is lifestyle modifications, however the introduction of drugs is taken into consideration<sup>[4]</sup>. Metformin is considered the initial pharmacological recommendation for the majority of type 2 diabetic patients either alone or in conjunction with other anti-diabetic drugs like insulin<sup>[5]</sup>. Numerous invitro studies proved that metformin can stimulate the osteogenic development of various mesenchymal stem cells

(MSCs)<sup>[6]</sup>. Metformin can also enhance odontoblast development and mineralization from dental pulp stem cells (DPSCs) which was evident by the expression of odontoblastic markers<sup>[7]</sup>. Based on the previous knowledge, this work aimed to study if in *vivo* metformin can prevent the adverse effect of GDM on tooth development and odontoblastic differentiation in offspring rats born from diabetic mothers.

# METHODOLOGY

#### Animal care and sample size

The experimental techniques followed a protocol authorized by the Ethical Committee of Faculty of Dentistry, Mansoura University, Egypt. With approval no A 10100221. The standard guides of the care and use of the laboratory animals were followed during handling of the animals.

For induction of pregnancy, 20 adult female and 10 adult male sprague-dawley albino rats weighing 180-200 g were selected. The animals were housed in standard

conditions at MERC (Medical Experimental Research Center) in Mansoura (Egypt). The animals were allowed to acclimatize for three weeks at a temperature of 26-28°C , standard humidity 65-70% and 12hr dark-light cycle accompanied by free accessibility to a standard soft high-carbohydrate diet, protein feed 23% and water.

Sample Size was calculated using PASS (power analysis and sample size software, version 15, 2017, Kaysville, Utah, USA: NCSS, LLC). To produce six offspring rats per each sacrification time, a total of 36 offspring rats are needed.

# Induction of pregnancy and grouping

The female and male rats (at a 2:1 conception rate) were allowed to mate overnight on the evening of the proestrus stage of the estrous cycle. The female rats were free of diabetes mellitus (the glucose serum level should be less than 7.0 mmol/L). The copulation was verified on the following morning by microscopic evaluation of the vaginal smear and the detection of sperms, or by the appearance of a vaginal or mucous plug. It is considered day zero of pregnancy. Subsequent labeling and separation of the pregnant rats was performed. One week following mating, the unpregnant rats were excluded<sup>[8,9]</sup>.

Twelve pregnant female rats were subsequently divided into three groups (four rats each); Group I (Non-diabetic, control): The pregnant rats were given an equal volume of citrate buffer solution only<sup>[10]</sup>. Group II (Diabetic): The pregnant rats were subjected to induction of GDM on the first day of pregnancy<sup>[9]</sup>. Group III (Diabetic + Metformin): The pregnant rats were subjected to induction of GDM. After GDM model was successfully established, treatment with metformin 50 mg/kg body weight began from the 4th day of pregnancy until birth (the average gestation time is 21 to 23 days). It was taken once daily, using an oral cannula or gavage<sup>[11]</sup>.

#### Induction of Gestational Diabetes Mellitus (GDM)

Eight pregnant rats were fasted beginning on the evening of day zero of pregnancy and lasting roughly 12 hours<sup>[12]</sup>. On the 1st day of pregnancy, they received a single intra-peritoneal injection of freshly prepared streptozotocin (STZ) (Sigma Chemicals Co., St. Louis, MO, USA) dissolved at a dose of 45mg/Kg body weight in 0.1 M cold sodium citrate buffer (pH 4.5)<sup>[9]</sup>. The buffer should be prepared freshly for immediate use within 5 min. Group I (non-diabetic, control) received an equal amount of citrate buffer<sup>[10,12]</sup>.

On day 3 of pregnancy, 48 h after STZ injection, the diabetic status was identified by measuring the fasting blood glucose level in pregnant female rats using a Mini Glucometer (One Touch Ultra) with a blood drop acquired from a puncture in the tail vein. Diabetic status was confirmed in pregnant female rats when the value of the fasting blood glucose was over 200 mg/dL, as approved by the WHO<sup>[13]</sup>, or blood glucose levels were found ranging from 13.9–22.2 mmol/L<sup>[14]</sup>. Then, metformin

administration begins from day 4 of pregnancy, the next day of diabetes confirmation.

# Regrouping After Measurment of Maternal Fasting Glucose level

On day four of pregnancy, weighing of all pregnant females was performed followed by measurement of fasting blood glucose level before initiation of the treatment. Then, the pregnant diabetic female rats having lower level of glucose were positioned in the diabetic untreated group, while those with high blood glucose levels were allocated to metformin group.

Regrouping was essential in avoiding early death of the pregnant diabetic untreated rats because rats with high diabetes rarely live long without therapy<sup>[15]</sup>. In addition, collecting the extremely diabetic rats in the drug-treated group will augment the effect of metformin<sup>[16]</sup>.

### Specimen Processing and Assessment Methods

Thirty-six offspring rats were obtained from three groups (12 from each group). A hand lens was used to examine each fetus for any morphological abnormality. At 10 and 21 days after birth, six rats from each group were euthanized via injection of a mixture of xylazine (5mg/ kg) and ketamine (75mg/kg) intraperitoneally at 10 and 21 days after birth<sup>[17]</sup>. After harvesting the animals, the heads of all three groups were fixed for 48 hours in 10% neutral buffered formalin. Then, they were conveyed to 14% neutral buffered ethylene diamine tetra acetic acid (EDTA) for proper decalcification after convenient washing under running tap water for about 30 min<sup>[18]</sup>. After decalcification, the specimens were stained with hematoxylin and eosin (H&E) according to Cardiff et al. (2014) protocol<sup>[19]</sup>, and anti-Nestin antibody [(MAB353) mono clonal rat-401, Sigma, St. Louis, MO, USA], a specific marker of odontoblast, according to the protocol done by Nakatomi et al.<sup>[20]</sup>. The slides were numbered and examined blindly. Finally, digital morphometry was carried out for the root length and Nestin expression, then results and data were statistically analyzed.

#### **Digital Morphometric Analysis**

At the maxillary first molar area ten fields in each group were examined and histological photographs were taken at 100X and 200X, then they digitized with a Toup Tek Toup view ® digital camera (model no.: XCAM1080PHA) mounted on an Olympus® microscope (CX22, Japan).

For root length measurement, analysis of the photographs was performed using Video Test Morphology® software (Russia) having a specific built-in routine for distance calibration. Measurements were performed using free hand line tool for measurement.

For analysis of Nestin expression: photographs at 200X were analyzed using VideoTest Morphology® software (Russia).

# Statistical Analysis

IBM-SPSS software was used for data analysis (IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp). Shapiro-Wilk's test was initially used to test normality, normally distributed data were considered if p>0.050. Boxplots tested the presence of extreme values. Quantitative data were presented as mean and standard deviation.

The Independent Samples T-test was utilized in comparison of normally distributed quantitative data between two groups (two time periods) and the one-way ANOVA test was utilized to compare normally distributed quantitative data between more than two groups, followed by the post-hoc Tukey test. Considering results statistically significant if the *p*-value was  $\leq 0.050$ .

# RESULTS

#### Hematoxylin and Eosin Results

#### 10 days after birth

The maxillary first molars tooth germs of albino rats of all groups were at the late bell stage of tooth development (Intra Osseous Phase of Eruption). (Figure 1) The control non-diabetic group revealed the normal structure of the maxillary first molar tooth germ with normal cellular appearance with regular arrangement and normal adhesion. (Figures1A,2A,2B) The enamel was absent in the cusp region of some specimens with short ameloblasts found only in this area directly attached to the underlying dentin. (Figure 2A) The root length (dash line) is determined from the cervical end of the enamel till the epithelial diaphragm (ED) ( $425\pm15.14$ ). (Figures 4A,6, Table1).

Group II (Diabetic, non-treated group) revealed some evident signs of degeneration of the maxillary first molar tooth germ. The ameloblast and odontoblast cells exhibited some disturbances in their arrangement with loss of homogenous continuity as they were detached in some areas from the underlying enamel and dentin matrices. There was also loss of cell-to-cell adhesion, with empty spaces (vacuoles) and some eosinophilic areas infiltrating the odontoblastic layer. (Figures 1B,2C,2D) Some odontoblastic nuclei appeared crescent shape. (Figure 2D) The length of the formed root was short (43.83±2.23) (Figures 4B,6,Table1).

Metformin treated group exhibited evident signs of normal development of the maxillary first molar tooth germ. Ameloblasts and odontoblasts revealed almost their normal cellular structure, with improvement in their arrangement, attachment, and physiologic adhesion between each other. (Figures 1C,2E,2F) The deposited dentin matrix and predentin showed slightly higher thickness than the diabetic group in the cervical region of the crown (Figure 4). There was an obvious progress in the root development denoted by the increased length of the developing root (409.8  $\pm$ 22.07) (Figures 4C,6, Table1).

#### 21 days after birth

The control non-diabetic group displayed the normal structure of the tooth germ of the maxillary first molar. Ameloblasts no longer appeared due to the eruption of the developing upper first molar in the oral cavity. The enamel was completely formed and fully mineralized proved by its complete absence after the decalcification leaving enamel space limited by the smooth gingiva. Odontoblasts revealed a pseudostratified columnar arrangement, with their nuclei appearing in more than one level. (Figures 1D,3G,3H) The developing root showed continuous formation, epithelial diaphragm and an adequate normal length (1375.8± 98.85) (Figures 4D,6,Table1).

The diabetic group showed delayed tooth eruption and root formation of the maxillary first molar. Ameloblasts were detected as a part of reduced enamel epithelium surrounding the enamel space. The pseudo stratification of the odontoblasts did not obviously appear, the cells were disorganized, demonstrating signs of degeneration with lack of cell-to-cell adhesion (empty spaces or vacuoles), and some eosinophilic areas and crescent nuclei were observed. (Figures 1E,3I,3J) The root development was delayed as the developing root appeared shorter (165.67  $\pm$  10.09) (Figures 4E,6Table1).

The treated diabetic group demonstrated signs of normal development of the tooth germ of the maxillary first molar on the cellular and eruptive level. The cells were normally arranged with a normal appearance, and the tooth erupted in the oral cavity by merging between the reduced enamel epithelium and the oral epithelium (Figures 1F,3K,3L). The developing root showed continuous formation with adequate normal length (908±13.74) (Figures 4F,6, Table1).

# Immunohistochemical Results (Nestin Expression)

#### 10 days after birth

Nestin showed a cytoplasmic immunoreactivity observed in odontoblast cells, odontoblastic processes penetrating dentin matrix tissue, and in developing pulp tissue, mainly the subodontoblastic layer. The normal mean % area of Nestin expression was  $14.138\pm0.05$ . The diabetic group exhibited a significant fall in Nestin expression in comparison to the control group, with a mean % area of Nestin expression  $5.09\pm0.05$ . After treatment with metformin, the expression started to rise reaching  $11.45\pm0.13$  (Figures 5,7, Table 2).

# 21 days after birth

The control group showed an increased Nestin expression after 21 days with a mean % area of  $22.873\pm0.06$ , while the diabetic group showed a significant decrease in Nestin expression compared to both the control group and the same group at 10 days with a mean % area of  $3.823\pm0.06$ . A significant increase in Nestin expression was noticed in the metformin treated group in comparison to the diabetic group with a mean % area of Nestin expression of  $18.402\pm$ 0.013 (Figure 5,7, Table 2).



**Fig. 1:** Photomicrograph showing the eruption stage in the study groups at different periods. All the teeth germs at 10 days were not erupted (A, B&C). After 21 days the teeth germs of the control and treated groups were erupted (D&F), while the diabetic group was not erupted (E). (H&E X40, Bar =  $250 \mu m$ )



Fig. 2: Photomicrograph showing the ameloblast and odontoblast cells of the study groups at 10 days after birth. The ameloblasts were normally arranged on the enamel of control group with localized shortening (arrow head) and abscence of enamel on the cusp tip (A). Normally arranged odontoblast cells (OB) (B). Ameloblasts of the diabetic group with elliptical nuclei (arrow head), loss of adhesion (arrow), and some area of detachment from the underlying enamel matrix (curved arrows) (C). Detached odontoblasts appeared with eosinophilic areas (tailed arrows), vacuoles (v), and crescent shape nuclei (wavy arrows) (D). Ameloblasts (AB) of the treated group were arranged on a thick enamel matrix layer in a homogenous continuous row (E). Odontoblast cells with normal appearance and arrangement (F). (H&E X400, Bar =  $25 \mu m$ )



Fig. 3: Photomicrograph showing the odontoblast cells of the study groups at 21 days after birth. Odontoblasts (OB) of the control group were arranged in a single homogenous layer with good adhesion to the predentin (PD) (G&H). Disorganization of the odontoblasts in the diabetic group with discontinuity and tearing (double arrow heads), detachment from underlying dentin (curved arrows), separation between the cells (corner arrows), vacuoles (v), some esinophillic areas (tailed arrows), and crescent nuclei (wavy arrows) (I&J). Odontoblasts of the treated group were arranged in a single homogenous layer attached to the underlying predentin (PD) with minimal vacuolization and elongated nuclei at the proximal ends (K&L). (H&E X100, Bar = 100  $\mu$ m) & (H&EX400, Bar= 25  $\mu$ m)



Fig. 4: Photomicrograph showing the length of the developing root (dash line) in different study groups at different periods. Deficient root length of the diabetic group in both tested periods (B&E) in comparison with the control (A&D) and treated group (C&F). (H&E X100, Bar=100  $\mu$ m)



**Fig. 5:** Photomicrograph showing Nestin expression in all study groups at different periods. Negative control slide of anti Nestin (A). Nestin expression in odontoblastic cell layer with their processes infiltrating dentin matrix tissue (arrow heads) (B, E, D&G). Sever deficiency of Nestin expression in the diabetic group (C&F) in comparison with the control group (B&E) and the treated group (D&G). (Anti-Nestin X200, Bar=  $50 \mu m$ )





Fig. 6: Clustered bar chart for mean of Root Length in different groups at different periods of time.

Fig. 7: Clustered bar chart for mean of Nestin positively stained area % for different groups at different periods of time.

Table 1: C	omparison	of root le	ength in	the three	groups an	d two time	periods.
------------	-----------	------------	----------	-----------	-----------	------------	----------

Time period	Group						
	Control		Diabetic		Treated diabetic		P2-value
	Mean	SD	Mean	SD	Mean	SD	_
10-days	425.0	15.14	43.83	2.23	409.8	22.07	<.001*
21-days	1375.8	98.85	165.67	10.09	908.00	13.74	<.001*
P1-value	<.001		<.001		<.001		

Notes: The test of significance is one-way ANOVA (P2-value) and independent-samples t-test (P1-value).

**Table 2:** Nestin expression in the three groups and two time periods.

	Group						
Time period	Control		Diabetic		Treated diabetic		P2-value
	Mean	SD	Mean	SD	Mean	SD	—
10-days	14.14	0.05	5.09	0.05	11.45	0.13	<.001
21-days	22.87	0.06	3.82	0.06	18.40	0.13	<.001
P1-value	<.001		<	<.001		<.001	

Notes: The test of significance is one-way ANOVA (P2-value) and independent-samples t-test (P1-value).

#### DISCUSSION

Due to the consistent correlation between diabetes and pregnancy, a clinical class known as gestational diabetes has been identified. This class comprises pregnant patients whose diabetes begins during the gestational period<sup>[21]</sup>. Pregnancy-related hyperglycemia is known to negatively impact fetal development, raising the possibility of defects in the dental enamel organ at this time and leading to abnormalities in the deciduous teeth of children born to diabetic mothers<sup>[22]</sup>.

Several drugs could be administered for experimental induction of diabetes like alloxan and STZ, or it could be induced surgically through pancreatectomy. Streptozotocin was utilized to induce diabetes because it possessed characteristics similar to those observed in human cases of diabetes, as well as its broad range of doses with the capacity to be administered in multiple low doses, and its very noticeable effect following intraperitoneal administration<sup>[23]</sup>.

The delivered dose of 45mg/kg was documented in a study to evaluate the influence of okra extract on GDM rats in which Tian *et al.* (2015) employed STZ in the production of GDM. They explained that in adult female rats, a single intraperitoneal dosage of 40 to 60 mg/kg body weight is the most often utilized dose for inducing diabetes mellitus<sup>[9]</sup>. Moreover, if the dosage is less than 40 mg/kg, it may be ineffective in induction of diabetes mellitus. For optimization of the results, STZ was preserved in a cold store (2-8°C) away from light with immediate preparation before injection, within less than 5 minutes. This is due to the instability of the solution and its reduced ability to induce diabetes if it is not used fresh<sup>[24]</sup>.

Over the past decades, insulin was the drug of choice for treating diabetes. However, several new oral hypoglycemic agents have been established. They have exceptional mechanisms of action that could be directed toward a definite organ<sup>[25]</sup>. Recent studies proved that metformin can provoke odontoblast differentiation and mineralization in *vitro*, in addition to having an obvious effect in treating

GDM without the production of toxicity signs such as hypoglycemia, renal toxicity and liver toxicity<sup>[26,27]</sup>. So, it was the drug of choice for our in *vivo* study to evaluate the effect of metformin on tooth development of offspring albino rats born from diabetic mothers.

The50mg/kg metformin was the dose of choice as all concentrations below this value couldn't affect odontoblast cell proliferation, as stated by Qin *et al.* (2018) who used metformin in stimulating the differentiation of dental pulp cells into odontoblasts<sup>[11]</sup>.

The developing rat molars provide an admirable model for studying epithelial morphogenesis. It has many advantages including simplicity in structure analysis which is almost similar to the human molar as they have roots, decreasing in size from before backward, and they are not of persistent growth<sup>[28]</sup>.

The euthanization of the rats at 10 days after birth was based on the fact that at this time, nearly all dental tissues were formed while being under the impact of maternal hyperglycemic status and began the calcification process, as reported by Silva-Sousa *et al* (2003)<sup>[29]</sup>.

The control group at 10 and 21 days after birth showed the teeth germs of maxillary first molars of rats' offspring in the intra-osseous (late bell stage) and extra osseous phase of the eruption, respectively with the normal cellular structure of odontoblasts and ameloblasts. These cells were arranged on a layer of dentin and enamel matrices of adequate thickness. The developing root exhibited normal development and a suitable length measured by histomorphometric analysis. These results were in agreement with Sanchez *et al.* (2020) who conducted a study to investigate the impact of exposure to chromium on dental tissues at different stages of tooth eruption<sup>[30]</sup>.

The diabetic group showed observable signs of tissue degeneration including disruption of the cellular integrity and continuity either in the ameloblasts or the odontoblasts cells with subsequent detachment of the underlying structures. The nuclei showed signs of degeneration as crescent shaped with vacuolization. A shorter length of the developing root was noticed during both periods. In addition, after 21 days, the teeth germs did not erupt into the oral cavity. These results could be explained by Chen et al. (2017), who declared that hyperglycemia exhibits various effects on the odontogenesis process in offspring tooth germ. It affects both the proliferation and apoptosis of dental mesenchymal and epithelial cell via enhancement of apoptosis and suppression of odontoblast cells proliferation through (TLR4/NF- KB) signaling pathway<sup>[31]</sup>, which is a pathway activated by maternal diabetes and crucially important in impairing embryonic development<sup>[32]</sup>. El-Ghawet et al. (2019) noticed also the decreased dentin thickness and the damage of some odontoblast cells in the offspring born from diabetic mothers. Widened vacuoles and eosinophilia between the odontoblasts were also detected by H&E. These changes were explained by the increased intensity of caspase

immunohistochemical expression denoting increased cellular apoptosis<sup>[33]</sup>. The damaging effect of diabetes on odontogenesis of the rat offspring could be attributed also to the occurred hyperglycemia during pregnancy that produced a defect in fetal  $\beta$  cells of the pancreas, and consecutively, a reduction in the level of insulin in fetuses, which led to a decline of the glucose transporter 1 (GLUT1) and glucose transporter 4(GLUT4) receptors<sup>[34]</sup>. The GLUT1 has a role in tooth growth and development as it is a protein required for the transportation of glucose as primary nutrition for the tooth germ of the developing fetus. Another explanation was due to the rise of oxidative stress as a result of the hyperglycemic state that leads to interruption of normal cellular function and deterioration of cellular lipid, protein and DNA<sup>[35]</sup>.

Treatment with metformin showed an improvement in ameloblasts and odontoblasts cellular integrity and arrangement, in addition to improvement in the root length and eruption state. Qotrunnada *et al.* (2021) had similar results since they found that offspring rats born from metformin treated diabetic mothers had accelerated tooth eruption with normal tooth growth and development. The teeth had entered the stage of apposition and calcification with the formation of HERS, and then the eruption stage, compared to offspring rats born from untreated mothers which were still in the late bell stage with no eruption. This may be attributed to metformin ability to improve blood glucose levels and insulin sensitivity during the period of development<sup>[36]</sup>.

In order to detect the influence of metformin on odontoblast cells and dentin regeneration, many in *vitro* studies have been done. They concluded that it would be difficult for the metformin to reach, in a systemic manner, the dental pulp stem cells (DPSCs) residing inside the tooth pulp enclosed by tooth structures<sup>[37]</sup>. However, in the present work, metformin proved for the first time its obvious systemic effect on odontoblast cell differentiation in addition to the other cells of the enamel organ of upper maxillary first molar born to diabetic pregnant mothers.

Staining with Nestin, a specific marker of odontoblasts<sup>[38]</sup>, proved that diabetes significantly decreases the amount of Nestin expression than the control group. Meanwhile, metformin treatment reversed the deleterious effect on odontoblastic differentiation and significantly increased the Nestin expression approximating the normal level in the control group. Moreover, there was a statistically significant increase in Nestin expression between the two tested periods (10, and 21 days) in the control and metformin groups, while in the diabetic group there was a statistically significant decrease in 21 days vs. 10 days.

This effect may be attributed to the significant upregulation of DSPP gene expression levels (important for specifying odontoblastic differentiation), DMP1, COL1 (an essential protein in dentin formation and repair), SP7 and RUNX2 (key factors essential for the proliferation and differentiation of odontoblasts)<sup>[7]</sup>. Other studies explained the metformin effect in the enhancement of odontoblastic differentiation of DPCs via the triggering and stimulation of the AMPK signaling pathway<sup>[11]</sup>.

# CONCLUSION

This study demonstrated that metformin can improve the degenerative effects of maternal gestational diabetes on offspring's odontogenic cellular differentiation, root formation and tooth eruption. It could also enhance Nestin odontoblastic marker expression. These results suggest that metformin may play an important pharmacological role in triggering odontoblastic differentiation. Therefore, it was advised that any pregnant woman with gestational diabetes take metformin to prevent adverse effects in development of her unborn child's teeth.

#### **CONFLICT OF INTERESTS**

There are no conflicts of interest.

## REFERENCES

- Saucedo R, Ortega-Camarillo C, Ferreira-Hermosillo A, Diaz-Velazquez MF, Meixueiro-Calderon C, Valencia-Ortega J. Role of Oxidative Stress and Inflammation in Gestational Diabetes Mellitus. Antioxidants (Basel). 2023;12(10).DOI: 10.3390/antiox12101812.
- 2. Taylor SI, Yazdi ZS, Beitelshees AL. Pharmacological treatment of hyperglycemia in type 2 diabetes. J Clin Invest. 2021;131(2).DOI: 10.1172/JCI142243.
- 3. Saghiri MA, Tang CK, Nath DJDR. Downstream effects from diabetes mellitus affected on various tooth tissues: A mini review: Effects of Diabetes on Tooth Structure. Dentistry Review.2021;1(1):100002.
- 4. Wu R, Zhang Q, Li Z. A meta-analysis of metformin and insulin on maternal outcome and neonatal outcome in patients with gestational diabetes mellitus. J Matern Fetal Neonatal Med. 2024;37(1):2295809. DOI:10.10 80/14767058.2023.2295809.
- Tocci V, Mirabelli M, Salatino A, Sicilia L, Giuliano S, Brunetti FS, *et al.* Metformin in Gestational Diabetes Mellitus: To Use or Not to Use, That Is the Question. Pharmaceuticals (Basel). 2023;16(9). DOI:10.3390/ ph16091318.
- Yin J, Lei Q, Luo X, Jiang T, Zou X, Schneider A, *et al.* Degradable hydrogel fibers encapsulate and deliver metformin and periodontal ligament stem cells for dental and periodontal regeneration. J Appl Oral Sci. 2023;31:e20220447. DOI: 10.1590/1678-7757-2022-0447.
- Lu J, Li R, Ni S, Xie Y, Liu X, Zhang K, *et al.* Metformin carbon nanodots promote odontoblastic differentiation of dental pulp stem cells by pathway of autophagy. Front Bioeng Biotechnol. 2022;10:1002291. DOI: 10.3389/fbioe.2022.1002291.

- De Souza Mda S, Sinzato YK, Lima PH, Calderon IM, Rudge MV, Damasceno DC. Oxidative stress status and lipid profiles of diabetic pregnant rats exposed to cigarette smoke. Reprod Biomed Online. 2010;20(4):547-552. DOI: 10.1016/j. rbmo.2010.01.002.
- Tian ZH, Miao FT, Zhang X, Wang QH, Lei N, Guo LC. Therapeutic effect of okra extract on gestational diabetes mellitus rats induced by streptozotocin. Asian Pac J Trop Med. 2015;8(12):1038-1042. DOI:10.1016/j.apjtm.2015.11.002.
- Abdul Aziz SH, John CM, Mohamed Yusof NI, Nordin M, Ramasamy R, Adam A, *et al.* Animal Model of Gestational Diabetes Mellitus with Pathophysiological Resemblance to the Human Condition Induced by Multiple Factors (Nutritional, Pharmacological, and Stress) in Rats. Biomed Res Int. 2016;2016:9704607. DOI: 10.1155/2016/9704607.
- Qin W, Gao X, Ma T, Weir MD, Zou J, Song B, et al. Metformin Enhances the Differentiation of Dental Pulp Cells into Odontoblasts by Activating AMPK Signaling. J Endod. 2018;44(4):576-584. DOI:10.1016/j.joen.2017.11.017.
- Adeniji AA, Lawal SK, Osinubi AA, Kusemiju T, Uyaiabasi N-FGJNQJoHM. The comparative effects of metformin and insulin on the kidney, lung and heart of streptozotocin-induced diabetic female Wistar rats. Journal of Hospital Medicine 2016;26(1):353-359.
- Shiming Z, Mak KK, Balijepalli MK, Chakravarthi S, Pichika MR. Swietenine potentiates the antihyperglycemic and antioxidant activity of Metformin in Streptozotocin induced diabetic rats. Biomed Pharmacother. 2021;139:111576. DOI:10.1016/j.biopha.2021.111576.
- 14. Zhou L, An XF, Teng SC, Liu JS, Shang WB, Zhang AH, et al. Pretreatment with the total flavone glycosides of Flos Abelmoschus manihot and hyperoside prevents glomerular podocyte apoptosis in streptozotocin-induced diabetic nephropathy. J Med Food. 2012;15(5):461-468. DOI: 10.1089/ jmf.2011.1921.
- 15. Lawal SK, Adeniji AA, Sulaiman SO, Akajewole MM, Buhari MO, Osinubi AA. Comparative effects of glibenclamide, metformin and insulin on fetal pancreatic histology and maternal blood glucose in pregnant streptozotocin-induced diabetic rats. Afr Health Sci. 2019;19(3):2491-2504. DOI: 10.4314/ahs. v19i3.25.
- Qinna NA, Badwan AA. Impact of streptozotocin on altering normal glucose homeostasis during insulin testing in diabetic rats compared to normoglycemic rats. Drug Des Devel Ther. 2015;9:2515-2525. DOI:10.2147/DDDT.S79885.

- Padial-Molina M, Marchesan JT, Taut AD, Jin Q, Giannobile WV, Rios HF. Methods to validate toothsupporting regenerative therapies. Methods Mol Biol. 2012;887:135-148. DOI: 10.1007/978-1-61779-860-3\_13.
- Kadashetti V, Shivakumar K, Baad R, Vibhute N, Belgaumi U, Bommanavar S, *et al.* A quantitative study of comparing the routine decalcification and microwave decalcification methods by using different decalcifying agents. Indian Journal of Multidisciplinary Dentistry. 2017;7(2):94.
- Cardiff RD, Miller CH, Munn RJ. Manual hematoxylin and eosin staining of mouse tissue sections. Cold Spring Harb Protoc. 2014;2014(6):655-658.DOI:10.1101/ pdb.prot073411.
- Nakatomi M, Quispe-Salcedo A, Sakaguchi M, Ida-Yonemochi H, Okano H, Ohshima H. Nestin expression is differently regulated between odontoblasts and the subodontoblastic layer in mice. Histochem Cell Biol. 2018;149(4):383-391. DOI: 10.1007/s00418-018-1651-3.
- 21. Chen G, Chen J, Yan Z, Li Z, Yu M, Guo W, et al. Maternal diabetes modulates dental epithelial stem cells proliferation and self-renewal in offspring through apurinic/apyrimidinicendonuclease 1-mediated DNA methylation. Sci Rep. 2017;7:40762. DOI: 10.1038/ srep40762.
- 22. Wei D, Loeken MR. Increased DNA methyltransferase 3b (Dnmt3b)-mediated CpG island methylation stimulated by oxidative stress inhibits expression of a gene required for neural tube and neural crest development in diabetic pregnancy. Diabetes. 2014;63(10):3512-3522. DOI: 10.2337/db14-0231.
- Gross GA. Drugs During Pregnancy and Lactation: Treatment Options and Risk Assessment. 2nd edition ed: Journal of the American College of Surgeons; 2008. p. 207.
- 24. Saad NM, El Masri NA, Shehata FI, Shafik S, El Adawy MMJIJ. Histomorphometric and Histological Evaluations of the Flaxseeds Effect on Dentin Formation in Offsprings' Teeth of Diabetic and Non-Diabetic Pregnant Rats. International Journal of Dental Sciences and Research. 2014;2(6C):1-7.
- 25. American Diabetes A. Addendum. 9. Pharmacologic Approaches to Glycemic Treatment: Standards of Medical Care in Diabetes-2020. Diabetes Care 2020;43(Suppl. 1):S98-S110. Diabetes Care. 2020;43(8):1979. DOI: 10.2337/dc20-ad08a.
- Gupta RC, Chang D, Nammi S, Bensoussan A, Bilinski K, Roufogalis BD. Interactions between antidiabetic drugs and herbs: an overview of mechanisms of action and clinical implications. Diabetol Metab Syndr. 2017;9:59. DOI: 10.1186/s13098-017-0254-9.

- Yamunadevi A, Pratibha R, Rajmohan M, Ganapathy N, Porkodisudha J, Pavithrah D, *et al.* Molecular Insight into Odontogenesis in Hyperglycemic Environment: A Systematic Review. J Pharm Bioallied Sci. 2020;12(Suppl 1):S49-S56.DOI:10.4103/jpbs. JPBS 159 20.
- Dammaschke T. Rat molar teeth as a study model for direct pulp capping research in dentistry. Lab Anim. 2010;44(1):1-6. DOI:10.1258/la.2009.008120.
- 29. Silva-Sousa YT, Peres LC, Foss MC. Are there structural alterations in the enamel organ of offspring of rats with alloxan-induced diabetes mellitus? Braz Dent J. 2003;14(3):162-167. DOI:10.1590/s0103-64402003000300004.
- Sanchez LM, Ubios AM. Alterations in odontogenesis and tooth eruption resulting from exposure to hexavalent chromium in suckling animals. Int J Paediatr Dent. 2020;30(1):35-41. DOI:10.1111/ ipd.12573.
- 31. Chen G, Sun W, Liang Y, Chen T, Guo W, Tian W. Maternal diabetes modulates offspring cell proliferation and apoptosis during odontogenesis via the TLR4/NF-kappaB signalling pathway. Cell Prolif. 2017;50(3). DOI: 10.1111/cpr.12324.
- Devaraj S, Tobias P, Jialal I. Knockout of toll-like receptor-4 attenuates the pro-inflammatory state of diabetes. Cytokine. 2011;55(3):441-445. DOI: 10.1016/j.cyto.2011.03.023.
- El-Ghawet H, El-Sayyad H, Ghanem N, Mkhemar SJJPCH. Cinnamon and ginger combined extract improved diabetic induced developmental defects of inner ear and teeth of rat fetuses. J Preg Child Health. 2019;6(411):2.
- Blackburn S. Maternal, Fetal, & neonatal physiology-E-book: a clinical perspective. 5th edition ed: Elsevier Health Sciences; 2017.
- 35. Ida-Yonemochi H, Nakatomi M, Harada H, Takata H, Baba O, Ohshima H. Glucose uptake mediated by glucose transporter 1 is essential for early tooth morphogenesis and size determination of murine molars. Dev Biol. 2012;363(1):52-61. DOI:10.1016/j. ydbio.2011.12.020.
- Qotrunnada S, Ummah DZ, Syafriadi MJMKG. Comparison of rat tooth eruption in rats born from diabetic mothers. Majalah Kedokteran Gigi. 2021;54(3):137-142.
- 37. Pradeep AR, Patnaik K, Nagpal K, Karvekar S, Ramamurthy BL, Naik SB, *et al.* Efficacy of locallydelivered 1% metformin gel in the treatment of intrabony defects in patients with chronic periodontitis: a randomized, controlled clinical trial. J Investig Clin Dent. 2016;7(3):239-245. DOI:10.1111/jicd.12150.

38. Terling C, Rass A, Mitsiadis TA, Fried K, Lendahl U, Wroblewski JJIJoDB. Expression of the intermediate filament nestin during rodent tooth development.

nternational Journal of Developmental Biology,. 2004;39(6):947-956.

الملخص العربى

# تأثير الميتفورمين علي تمايز الخلايا المكونة للعاج وتكوين الجذر أثناء نموأسنان ذرية الثير الميتفورمين علي تمايز الفئران المصابة بداء السكرى

ياسمين عادل صبري، أسماء صالح المهدي، فاطمة إبراهيم، ماهيتاب الجميلي قسم بيولوجيا الفم، كلية طب الأسنان، جامعة المنصورة

مقدمة : سكري الحمل هو فئة سريرية تشمل المرضى الحوامل المصابات بالسكري أثناء الحمل،ويؤثر سلبًا على نمو الجنين، مما يؤدي إلى عيوب في مينا الأسنان وتشو هات الأسنان اللبنية. الهدف: أجريت هذه الدراسة لتقييم تأثير الميتفورمين على تطور نمو الأسنان في ذرية الفئران المصابة بداء السكري. منهجية البحث: تم تحفيز الحمل في ١٢ فأرة بيضاء بالغة، يتراوح وزنها بين ١٨٠-٢٠٠ جرام، و بعد تأكيد اليوم الاول من الحمل، تم تقسيم إناث الفئر ان الحو امل إلى ثلاث مجمو عات (العدد =٤ لكل منها) كالتالي: المجموعة الأولى :(سليمة بدون مرض السكري): أعطت محلول السترات المتعادل فقط . المجموعة الثانية: تم تحفيز مرض سكرى الحمل في هذه الفئران في اليوم الأول من الحمل و تركت بدون علاج. المجموعة الثالثة: تم تحفيز مرض سكري الحمل في هذه الفئر ان و عُولجت بالميتفور مين، بجرعة ٥٠ ملغم/كغم من وزن الجسم - مرة واحدة يومياً- بدءاً من اليوم الرابع من الحمل حتى الولادة. تم تحفيز داء سكرى الحمل في المجموعة الثانية والثالثة، عن طريق حقن الستربتوزوتوسين، بمقدار ٤٥ملغم/كغم من وزن الجسم داخل الصفاق و التأكد من ارتفاع سكر الدم إلى أكثر من ٢٠٠ ملجم/ديسيليتر. التقييم الهستولوجي و الهستوكيميائي المناعي و الإحصائي: بعد اكتمال الحمل و الولادة، تم الحصول على سنة وثلاثين فأرأ مولوداً من المجموعات الثلاث (١٢ من كل مجموعة)، و تم التضحية بستة فئر ان من كل مجموعة بعد أيام من الولادة، وستة آخرين بعد ٢١ يومًا. بعد أخذ عينات الرؤوس و تطريتها، وضعت داخل مكعبات من الشمع، و قطعت على هيئة شرائح لكي تصبغ بالصبغات التالية: صبغة الهيماتو كسيلين والأيوسين صبغة نيستين الكيميائية المناعية للكشف عن مدي تمايز الخلايا المولده للعاج. ثم خضعت النتائج لتحليل الصور والتقييم الكمي، متبوعًا بالتحليل الإحصائي باستخدام اختبار ANOVA أحادي الاتجاه متبوعًا باختبار توكى اللاحق. النتائج: أظهرت النتائج أن مرض السكري يؤثر سلباً على عملية تكوين الأسنان، في الأجنة المولودة لأمهات مصابة بسكري الحمل، حيث لوحظت تغيرات انتكاسية في خلايا تكوين المينا والخلايا المكونه للعاج ، مصحوبة بتأخر في تكوين الجذر ومراحل ظهور الاسنان. وقد لوحظ انخفاض كبير في صباغة النيستين المناعية في مجموعة مرضى السكري. أما نتائج المجموعة الثالثة المعالجة بالميتفور مين ، فقد أظهرت حماية خلايا تكوين الأسنان من التغير ات السلبية التي ظهرت في المجموعة الثانية بسبب مرض السكري ، كما أظهرت أيضًا نمو طبيعي لجذور الأسنان وزيادة في صبغة النيستين المناعية الذي يعبر عن تمايز طبيعي للخلايا المكونة للعاج.

خلاصة البحث: اعتمادا على النتائج السابقة يمكننا استنتاج أن العلاج بالميتفور مين يخفف من التأثير ات الضارة لمرض سكري الحمل على تمايز الخلايا المكونة للعاج،والنمو الطبيعي لجذور الأسنان ،وعملية تكوين الأسنان و مراحل ظهور ها في ذرية الفئران البيضاء المولودة من الأمهات المصابة بالمرض.

وبالتالي هذه النتائج تقترح ان الميتفور مين ربما يلعب دورًا دوائيًا مهما في تحفيز التمايز الطبيعي لخلايا العصب السنية.