Effect of Diabetes during Pregnancy to Fetal Tooth Germ Growth and Development

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Abstract

Diabetes mellitus is a chronic metabolic disease characterized by hyperglicemia caused by pancreatic insulin production deficiency or ineffectiveness insulin produced. The objective was to determine the effect of fetal tooth germ growth and development disturbance due to diabetic during pregnancy. Five pregnant rats were induced by diabetes using 40 mg/kg b.w streptozotocin intraperitonealy and five pregnant normal rats as a control group. Pregnant rats with blood glucose level ≥ 200 mg/dl considered as diabetes. Blood glucose level was measured before, after induction, and just after birth. One rat offspring sample taken from each pregnant rats using simple random sampling and euthanized on 1st day postnatal. Rat offspring right maxilla taken to observe tooth germ growth and development. Paraffin-embedded tissue cut 4µm in thickness and stained using Haematoxylin-Eosin, Mallory's Trichrome and insulin-like growth factor 1 (IGF-1) Immunohistochemistry staining. Rat offspring who born from diabetic pregnant showed lower body weight which is statistically significant difference and histologically seen delayed of enamel matrix formation, tooth development stages, and also reduced in tooth size compared to control group. Therefore, seems different of IGF-1 expression in inner enamel epithelium tooth germ between two groups. Rat offspring who born from diabetic pregnant had tooth germ growth and development disturbance.

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Introduction

Diabetes Mellitus (DM) is a chronic metabolic disease characterized by hyperglicemia that disturb carbohydrate, protein and lipid metabolism caused by insulin production deficiency or ineffectiveness of the insulin produced.[1] Prevalence of DM increased per year worldwide exhibited the most increase in countries with emerging economy. This increase on DM prevalence due to urbanization and economic progress in emerging economy country.[2] Diabetes during pregnancy, also called gestational diabetes mellitus (GDM) can cause some negative effect to fetal tooth germ growth and development.[1]

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Diabetic hyperglicemia can promote reactive oxygen species (ROS) formation and develop oxidative stress that can cause damage to cellular lipids, protein, DNA and disturb normal cell function.[3] In diabetic hyperglicemia growth hormone (GH) levels are raised while insulin-like Growth factor-1 (IGF-1) levels are reduced.[4] Low levels of IGF-1 cause tooth germ growth and development disturbance, because IGF-1 has important role for cell longevity, protein synthesis, cell proliferation, decrease oxidative stress, and prevent cell death.[5, 6]

Materials and Methods

This study was experimental laboratories with the post-test only control group design. Ten wistar rat offspring (postnatal day-1) *Rattus norvegicus* from 10 different pregnant rats has been selected by using simple random sampling. Pregnant rats used for this study were about 13th day of pregnancy and were physically healthy with criteria of normal eyes, movement, behavior and good appetite feeding. All samples used for this study were divided to two group which are group 1 (control group) consist of 5 rats offspring

born from pregnant rats with normal blood glucose level (BGL) and Group 2 (diabetes group) consist of 5 rats offspring born from diabetic pregnant rats induced by streptozotocin (STZ) 40 mg/kg body weight intraperitoneally.

Glucose level of Pregnant rats measured by glucometer taken from tail lateral vein before diabetes induction (13th day of pregnancy), one day after induction, every 2 days and just after birth. Pregnant rats with BGL ≥ 200 mg/dl were considered as diabetes group[7]. Rat offspring body weight measured before euthanasia on 1st day postnatal.

All right maxilla of rat offspring samples taken and decalcified to prepare paraffin-embedded tissue processing. Each tissue samples cuted 4µm in thickness as many three slides for haematoxylin-Eosin (HE), Mallory's Trichrome and insulin-like growth factor 1 (IGF-1) Immunohisto-chemistry staining. All Histological staining observe under light microscope 100x and 400x magnification to see tooth development stage and size measuring by HE staining, observing enamel matrix formation by mallory's trichrome, and IGF-1 expression by Immunohisto-chemistry staining. Histo-morphological size of tooth germ of each samples measured 3 times by two observer using Image Raster application. Measuring tooth germ size performed at convex point of mesial inner enamel epithelium (IEE) to distal IEE to determine tooth germ size in-width, at servical to occlusal IEE to determine tooth size in-length and from occlusal IEE to occlusal OEE to determine stellate reticulum of tooth germ in-thickness. IGF-1 expression was calculate in IEE and dental papilla in 3 field of view (FOV) were 1/3 distal, 1/3 center, and 1/3 mesial of first molar tooth germ meanwhile IGF-1 expression interpreted by using ImmunoRatio web based application to count immuno-positive cells. All data statistical analyzed using Mann-Whitney if did not homogeneity and abnormal distribution and One Way Anova for homogeneity and normal distribution data.

Results

Group 2 (diabetes group) showed increasing BGL from 91±13.6 mg/dl to 410 ± 67.5 mg/dl after diabetes induction and after delivery reduced to 271 ± 66.9 mg/dl it is mean all the pregnant samples still in hyperglicemia condition (≥ 200 mg/dl). Therefore group 1 (control group), the BGL seem slightly increased after delivery from 91 ± 11.3 mg/dl to 93 ± 14.8 mg/dl (Table 1).

Sample	Before STZ Induction (13th day of pregnancy)	After STZ Induction (14th day of pregnancy)	After Delivery
C1	76 mg/dl	-	90 mg/dl
C2	83 mg/dl	-	107 mg/dl
C3	94 mg/dl	-	103 mg/dl
C4	105 mg/dl	-	94 mg/dl
C5	95 mg/dl	-	69 mg/dl
$\overline{X}$	91±11.3 mg/dl	-	93±14.8 mg/dl
D1	105 mg/dl	377 mg/dl	246 mg/dl
D2	82 mg/dl	461 mg/dl	333 mg/dl
D3	89 mg/dl	404 mg/dl	223 mg/dl
D4	72 mg/dl	319 mg/dl	201 mg/dl
D5	108 mg/dl	489 mg/dl	350 mg/dl
$\overline{X}$	91±13.6 mg/dl	410±67.5 mg/dl	271±66.9 mg/dl

Table 1. The Green Tea Extract Ability in Binding gp120 and gp41.

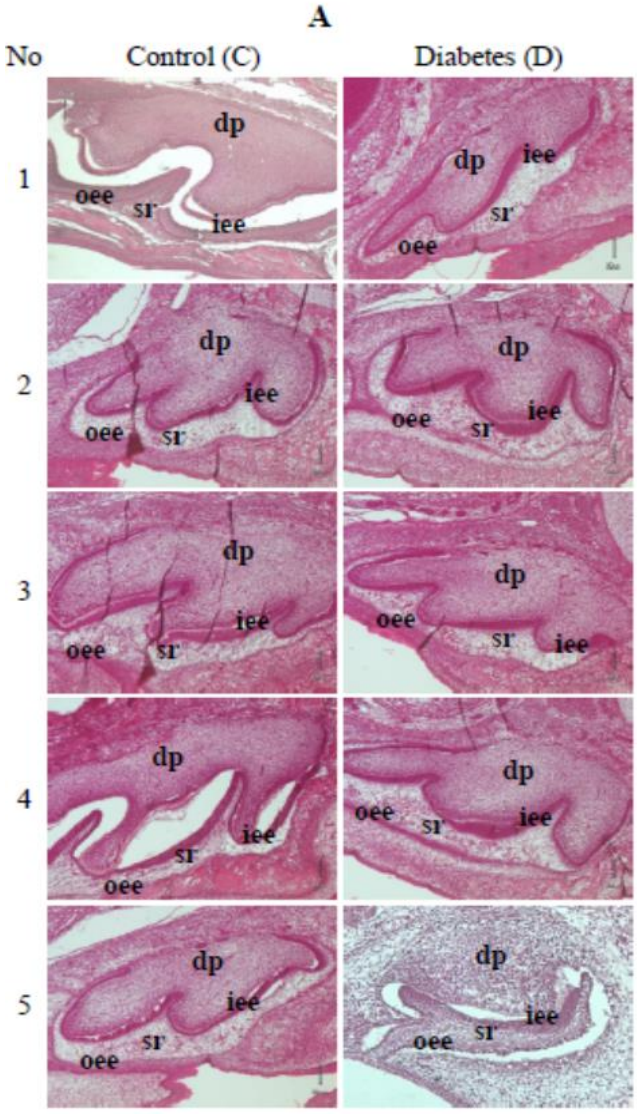
Increasing of BGL in group 2 impact to rat offspring average body weight compared to group 1 (control) that was 5 gr and 6 gr body weight consecutively. Statistical test showed there was significance difference between two group ($p < 0.05$) It is mean that rat offspring who delivery from diabetic pregnant rat had lower body weight than normal pregnant rat (Table 2).

Based on histological of tooth development showed that diabetes group had tooth development delay compared to control group. At Control group the first molar tooth germ had been shown advance bell stage in each sample, while diabetes group almost all samples seem bell stages, one sample still in bud stage of tooth development (Figure 1A).

According to histo-morphological tooth size, showed there was reducing tooth germ size both in-width and in-length of diabetes group compared to control group (Table 3) and statistically it was significant different ($p < 0.05$) meanwhile the thickness of tooth germ stellate reticulum showed no significant different seem between two group. Groups ($p > 0.05$).

Sample	Body Weight	$\overline{X}$
C1	6 gr	
C2	7 gr	
C3	6 gr	6 gr
C4	6 gr	
C5	7 gr	
D1	4 gr	
D2	5 gr	
D3	5 gr	5 gr
D4	5 gr	
D5	5 gr	

Table 2. Rat Offspring Body Weight of Control (C) and Diabetes (D) group.



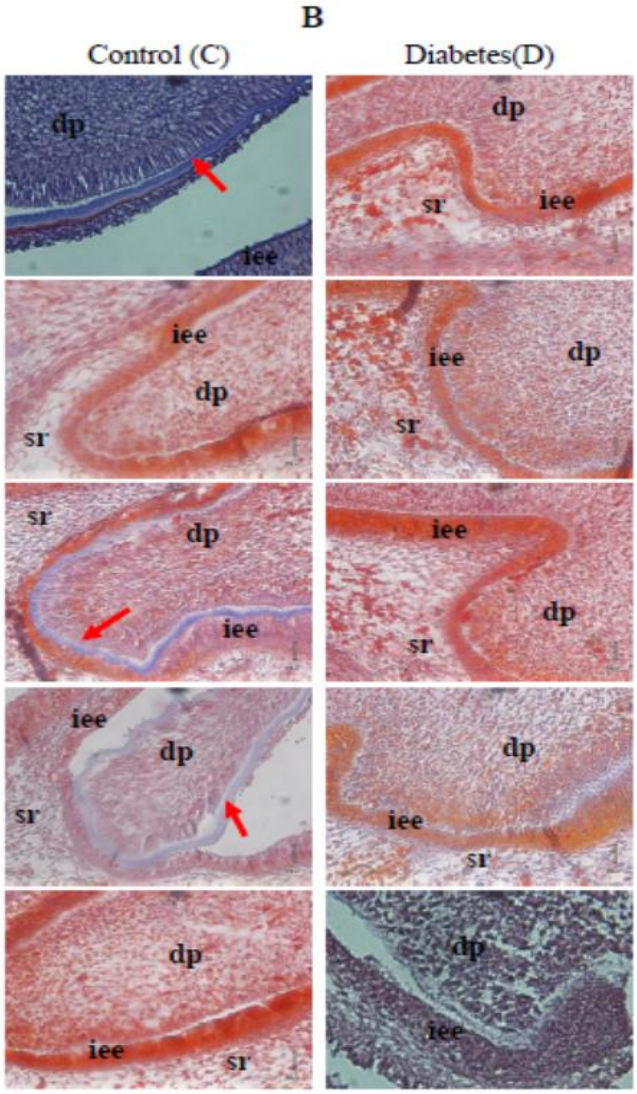


Figure 1. (A) Rat Offspring First Molar Tooth Germ Morphology Stained by HE 100x Magnification Showed Bell-like Shape Morphology in Each Sample of Control Group (C1, C2, C3, C4, C5),and in Sample (D1, D2, D3, D4) of Diabetes Group, Also Showed Cap-like Shape Morphology in Sample (D5) of Diabetes Group; (B) Mallory's Trichrome Staining 400x Magnification. Enamel Matrix was Formed (Red Arrow) in Control Group (C1, C3, C4) and Not Yet Formed in Diabetes Group. dp, Dental Papilla; iee, Inner Enamel Epithelium; oee, Outer Enamel Epithelium; sr, Stellate Reticulum.

Formation and mineralization of tooth germ enamel matrix in diabetes group also seem delayed compared to control group. Enamel matrix was formed and also mineralization had been done in almost of samples (C1, C3, C4) in control group even thought, 2 samples still not present matrix enamel, while all samples in diabetes group showed matrix and mineralization enamel not performed yet (Figure 1B), therefore that happen impact to tooth germ size.

	C1	C2	C3	C4	C5	$\overline{X}$	D1	D2	D3	D4	D5	$\overline{X}$	P
Mesio-Distal													
IEE to IEE*	701.9	563.5	699.2	828.8	598.1	678.3±104	506.6	480.5	581.1	587.7	246.1	480.4±138.9	0.033
Cervical-Oclusal IEE*	336.5	354.8	271.9	317.3	209.6	298.0±58.2	127.6	165.4	227.2	265.6	147.9	186.7±57.7	0.016
IEE to OEE	35.3	59.7	75.5	63.3	86.6	64.08±19.3	61.7	57.9	64.5	71.2	42.2	59.5±10.8	0.065

Table 3. Rat Offspring Tooth Size Measurement (µm).

	IGF-1 Expression in IEE	$\overline{X}$	IGF-1 Expression in Dental Papilla	$\overline{X}$
C1	97.90%		85.70%	
C2	100%		17.53%	
C3	54.45%	87.90±19.3%	15.00%	31.77±30.4%
C4	98.85%		15.70%	
C5	88.30%		24.90%	
D1	53.10%		10.30%	
D2	77.10%		13.76%	
D3	23.95%	61.56±28.2%	67.00%	32.66±28.6%
D4	54.70%		11.53%	
D5	98.95%		60.70%	
		p = 0.175		p = 0.347

Table 4. IGF-1 expression in IEE and dental papilla of control (C) and diabetes (D) group interpreted by ImmunoRatio web based application.

Rat offspring delivered from diabetes pregnancy group had lower IEE IGF-1 expression percentage compared to control group, otherwise IGF-1 expression in dental papilla slightly higher than control group (Figure 2). However statistically no significantly different between IGF-1 expression percentage both in IEE and dental papilla in those group ($p > 0.05$). The average of IEE IGF-1 expression in control group was 87.90 ± 19.3%, while diabetes group expression was average 61.56 ± 28.2% and average of IGF-1 expression at dental papilla was 31.77 ± 30.4% in control and 32.65 ± 28.6% in diabetes group (Table 4).

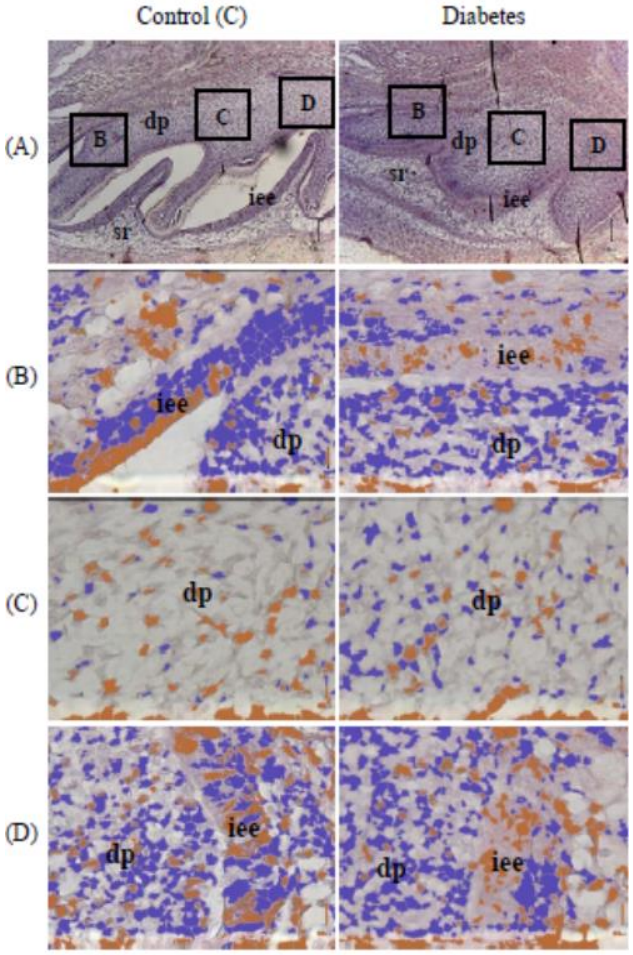


Figure 2. IGF-1 Immunohistochemistry staining 100x magnification, 1000x magnification in 1st FOV, 1000x magnification in 2nd FOV and 1000x magnification in 3rd FOV showed IGF-1 expression in IEE and dental papilla was brown colored. dp, dental papilla; iee, inner enamel epithelium; oee, outer enamel epithelium; sr, stellate reticulum.

Discussion

Increasing BGL in diabetes group caused insulin secretion disturbance by carbohydrate metabolism disturbance and hyperglycemia. Diabetes group pregnant rats insulin secretion disturbance were cause by STZ induction that comes from *Streptomyces achromogenes* which is toxic to islet langerhans β cell of pancreatic gland. STZ was commonly used for islets of langerhans cancer treatment and to make hyperglycemia condition on experimental animals during medical research.[8,9] STZ causes pancreas β cell damaging by DNA methylation, also decreasing NAD+ and free radicals producting, it had been effect to decreasing insulin secretion. By using STZ agent in this experimental also showed increasing BGL until ±410 mg/dl at 1st day after induction and all samples was hyperglycemia condition until 1st day postnatal, even thought, all samples in diabetic group BGL decrease after delivery but it still higher than normal BGL (>200 mg/dl). STZ could destroy pancreas β cells in the beginning but the cells have good regeneration capability.[8] That way in this study showed BGL decreasing from 1st day induction until 1st day delivery.

Rat offspring who born form diabetes pregnant group had lower body weight compared to control group and that difference of the body weight statistically significance difference between two groups. This result prove that hyperglycemia during pregnancy has significance effect to rat offspring body growth and development. Giavini *et al*. and Soldado & Harrera also reported that diabetes during pregnancy caused fetal hypo-insulinemia to rat offspring led to fetus glucose metabolism disturbance and fetus malnutrition.[10,11]

Damaging pregnant rat pancreas β cell that led diabetic hyperglycemia caused increasing fetus BGL by maternal blood transfer through placenta resulting fetus pancreas β cell become actively and bigger (hypertrophy) to compensate and blood fetal hyperglycemia. Fetus pancreas β cell adaptation to high BGL lead to over produce and secret insulin those it caused hyperinsulinemia, that hyperactivity of pancreas β cell producing insulin just temporary and can cause pancreas β cell which were not fully develop exhausted and damaged. Exhausting and damaging Fetus pancreas β cells lastly causes hypoinsulinemia and decrease on rat offspring body weight.[12]

Histological appearance of diabetes group rat offspring first molar in this study showed growth and development disturbance, such as delayed stages of tooth development might cause delayed proliferation and differentiation ameloblast cells and resulted delay enamel matrix formation. Those happen may explain that hyperglycemia that occurred during pregnancy caused fetal β cells pancreas defect sequentially decrease insulin level in fetal lead to decrease glucose transporter 1 (GLUT1) and glucose transporter 4 (GLUT4) receptors on bone including at tooth development and muscle tissue but opposite that on the vital organ such as brain occur increasing of GLUT1 because that tissue need more nutrition due to brain activity cells.[13, 14] Yonemochi *et al*. study was showed that GLUT1 important role in tooth growth and development, because GLUT1 was protein that needed to transport glucose as primary nutrition for developing and size determine fetus tooth germ.[15]

Rat offspring tooth germ growth and development disturbance of diabetes group also caused by increased of oxidative stress due to hyperglycemia. Increasing on oxidative stress may cause damage to cellular lipid, protein and DNA, also disturb normal function of cell such as tooth germ cells.[16]

Increasing of oxidative stress in hyperglicaemia by lipid and protein glycation process pathway which results increasing of AGEs production. AGEs product produced by Maillard reactions which is characterize by present of the alkylated amino acids, fluorescence residues, intra molecular and inter molecular cross linkage. AGEs has significance roles of diabetes complication, both intracellular AGEs and extracellular AGEs. Interaction between AGE and RAGE will increase intracellular ROS production and caused increase on oxidative stress.[17] In other way oxidative stress in hyperglicaemia resulted from polyol and protein kinase C pathway activation that leads decreasing of NADPH and increasing oxidative stress.

Normally most of intracellular glucose metabolism by phosphorylation and glycolysis pathway, however under hyperglicaemia condition glucose had been converted to sorbitol by aldose reductase. Intracellular osmolarity will increase due to increasing in intracellular sorbitol and was responsible for NADPH decrease and increase in oxidative stress.[18,19]

Severe tooth germ growth and development disturbance showed in diabetes group sample D5 which was still in cap stage on day 1 post-natal. It happen occurred because sample D5 delivered form maternal pregnant rat who had highest BGL compare to other maternal pregnant rat in the same group. This severe hyperglicaemia causes severe oxidative stress and fetus malnutrition. Severe hyperglycaemia as an indicator of body incapability to metabolize carbohydrate, that really important for tooth germ growth and development.[20]

Delayed of tooth germ growth and development also influenced by IGF-1 that was important to tooth germ growth and development. Bio-cellular activity of IGF-1 can maintain cell longevity by prevent cell apoptosis and promote cell proliferation by Akt pathway that activate 3 main protein which were mTORC1, FOXO and GSK3. Activation of mTORC1 promote protein synthesis, while activation of FOXO and GSK 3 inhibit protein degradation and apoptosis. IGF-1 also has important role in protein synthesis for bone matrix formation and determine body composition. Increasing of growth hormone (GH) and decreased of IGF-1 in blood circulation happened under hyperglicaemia diabetic condition that caused by hypoinsulinemia. As known that insulin is essentially needed for hepar organ to secrete IGF-1[21]. It was been proved in this study IGF-1 expression were lower in rat offspring tooth germ IEE who born from diabetic group.

Low of IGF-1 expression in IEE tooth germ derived from hyperglicaemia of maternal pregnant rats to her fetus through plasenta lead to fetal hipoinsulinemia also hyperglicaemia condition stimulate increasing GH circulation, both of them cause insensitive of hepar organ to GH. Growth hormones can't interact with GH receptors that effect to inhibiting of IGF-1 secretion.[21] IGF-1 secreting by hepar is really affected by GH, nutrition, BGL, physical activity, stress, age and gender.[22]

Expression of IGF-1 in dental papilla rat offspring tooth germ in diabetes group slightly higher than control group even though it was statistically not significant. It perhaps caused by IGF-1R in dental papilla rat offspring tooth germ cells in control group less than diabetic group due to their differentiation and maturation of dental papilla mesenchymal cells, so IGF-1R expression in control group was weaker. Normally, IGF-1R expression is reduce according to increasing of differentiation and maturation of dental papilla mesenchymal cells, meanwhile IGF-1R expression in IEE of rat offspring tooth germ is not affected by differentiation and maturation. IGF-1R expression in IEE same strong expression in early and advance stage of germ growth development.[23] In this study we found that IGF-1 expression in IEE weaker than in dental papilla in tooth germ of rat offspring had been born from diabetes group may perhaps effect of hipoinsulinemia.

Atreja *et al*., 2012 reported that IGF-1 plays an important role in GH bio-cellular activity. GH deficiency was well known has negative effect to tooth growth and development. GH deficiency can cause delayed tooth eruption and tooth apical shorten. GH deficiency also can disturb dental hard tissue matrix formation leads to amelogenesis imperfecta and in rare case GH deficiency can cause tooth agenesis.[24] According to Litsas, 2015 reports that reducing of IGF-1 levels can cause reduction in mesio-distal of tooth size.[25] It is same to this study that rat offspring tooth germ size born from diabetic group had small size compare to control group in width (mesio-distal) and length (cervico-occlusal).

Conclusion

Rat offspring who born from diabetic pregnant had lower body weight and had tooth germ growth and development disturbance, such as tooth size reduction, delayed of differentiation and maturation (stages) that lead to delayed of enamel matrix formation. Rat offspring who born from diabetic pregnant also had low IGF-1 expression on IEE due to fetal hipoinsulinemia.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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