

# Lipopolysaccharide (LPS) introduction during growth and development period of rat's tooth toward the occurrence of enamel hypoplasia

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## ABSTRACT

The aim of this study is to know the effect of lipopoly saccharide (LPS) induction during growth and development period specifically the occurrence of hypoplasia on tooth enamel. 5 day old male wistar rats divided into two groups. Group 1 (control) under went no treatment. Group 2 (treatment) under went LPS induction every 24 hour for 8 days on buccal fold right maxillary first molar. After 21 days old the rats were sacrificed and the tooth was resected. Hypoplasia Hypo calcification Index (HHI) was used to determine the degree of hypoplasia by clinical examination. Radiograph of maxilla was also taken to analyze the apacities of enamel by using COREL DRAW version 11. The result showed that group under went LPS induction hypoplasia occurred on its molar tooth and more radiolucent than control groups. The conclusion is LPS induction during growth and development period of rats tooth causing enamel hypoplasia.

**Key words:** lipopolysaccharide, growth and development tooth, hypoplasia, rat

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## INTRODUCTION

Growth and development of tooth consist of several stages; complex process and involve coordination as well as interaction between neural crest and epitel.<sup>1</sup> The stage and process consist of initiation (bud stage), proliferation (cap stage) histodifferentiation and morfodifferentiation (bell stage), apposition as well as calcification (mineralization). The change occurring during the period of growth and development would contribute tooth abnormality. The change might be resulted from various kind of local and systemic factors.<sup>2</sup>

One of local factors inducing tooth abnormality is bacterial infection during the period of tooth growth and development. Periapical lesion and alveolar bone impairment affected by either bacteria or product of other bacteria, one of them is lipopolysaccharide (LPS). LPS is the main structure of cell wall of gram negative bacteria to maintain the integrity of bacterial structure and to protect the bacteria from host immunity. In addition, LPS is endotoxin contributing the occurrence of cytokine proinflammatory secretion from several types of cell.<sup>3</sup> It could be suggested that LPS has an important role as the cause of periapical lesion.<sup>4</sup> This condition would induce local factor cytokine proinflammatory such as: interleukin -1 (IL-1), IL-1, IL-6, tumor necrosis factor- (TNF-) and eikosanoid i.e. prostaglandin (PGE<sub>2</sub>).<sup>5</sup> Prostaglandine and cytokine proinflammatory might result distruction of periodontal tissue by stimulating the formation and the increase of osteoclast activities.<sup>6</sup>

Enamel hypolasia is one of abnormalities of tooth enamel which frequently found in children's teeth caused by the disturbance during the period of tooth growth and development. In general, clinical feature of enamel hypoplasia is shown by enamel agenesis which looks like a small well, horizontally arranged found on buccal region. If severe hypoplasia occurs, most parts of tooth enamel will disappear.<sup>7</sup> Hypoplasia is called as quantitative abnormality, usually followed by hypocalcification which is called qualitative abnormality.<sup>8</sup> Hypocalcification (demarcated opacities) resulted from imperfect mineralization on enamel and manifest in the form of white spot.<sup>7</sup> Li *et al.*<sup>9</sup> reported that enamel hypoplasia of children's permanent teeth in rural area in China suspected caused by continuous caries on deciduous teeth which initially happened at the age of 3-5 years. Study conducted by Lo *et al.*,<sup>10</sup> Nicolau *et al.*,<sup>11</sup> and Broad Bent *et al.*<sup>12</sup> suggested that demarcated opacities and hypoplasia which happened in permanent tooth due to severe caries on the previous deciduous teeth even though the mechanism was still unknown. The purpose of this study is to know the effect of LPS induction enamel hypoplasia. The outcome of the study is expected to be able to contribute scientific in formation on the mechanism of hypoplasia, so that local infection which could disturb the growth and development could be avoided. In addition, it is also expected as the basic guidance of further study in the future.

## MATERIAL AND METHOD

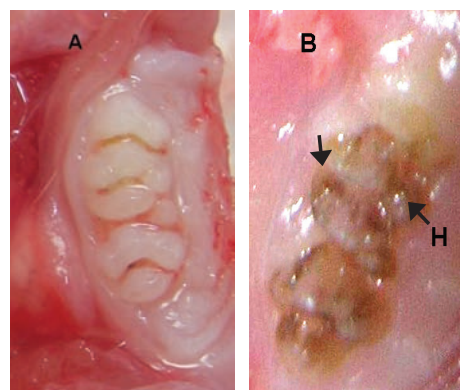
This study was performed on 10 male wistar rats of 5 days old, the rats were classified into 2 groups (5 rats each): groups 1: was controlled group which didn't undergo any treatment, groups 2: the rats were induced using *Escherichia coli* LPS (sigma) with the dosage: 5 gr/0,005 ml saline on buccal fold of right maxillary first molar. LPS induction was done by intra muscular injection once in 24 hours for 8 days.<sup>13</sup> On the 21<sup>st</sup> day the rats were decapitated. The maxilla was observed to find whether hypoplasia occurred or not enamel of right molar. The degree of hypoplasia was assessed by hypoplasia hypocalcification index (HHI)<sup>14</sup> occurrence or hypocalcification, as follows 0 = no hypoplasia occurrence or hypo calcification, 1 = hypocalcification on half of incisal or occlusal crown, 2 = hypocalcification on half of cervical crown, 3 = hypoplasia on half incisal or occlusal crown, 4 = hypoplasia on half of cervical crown, 5 = hypocalcification less than half incisal occlusal crown, 6 = hypocalcification more than half of incisal occlusal crown or affected more than one crown, 7 = hypoplasia less than half of incisal occlusal crown, 8 = hypoplasia more than half insisal occlusal or affected more than one occlusal, and 9 = hypocalcification/hypoplasia excluded the above criteria (disfusive hypoplasia, limited one occlusal expected incisal).

The result of radiopaque was put on x-ray viewer, then, recorded by digital camera and the result was transferred into computer. Radiopaque and radiolucent feature of tooth enamel was observed using COREL DRAW version 11 (Corel, Ottawa Canada) assessing the size of red blue green (RGB) to see the difference of radiopaque of every radiopaque.<sup>15</sup> The combination of RGB would produce white color, so, it is called additive color. The computer monitor would produce color shining ray through RGB. The mean of the total component of RGB would come maximal score = 255, and it would show pure white while the mean of total RGB would come: 0, it would show pure black. Statistic analysis would be done using T-test in order to know whether there is or there is no statistical difference between the two groups either based on HHI radiopaque of radiopaque.

## RESULT

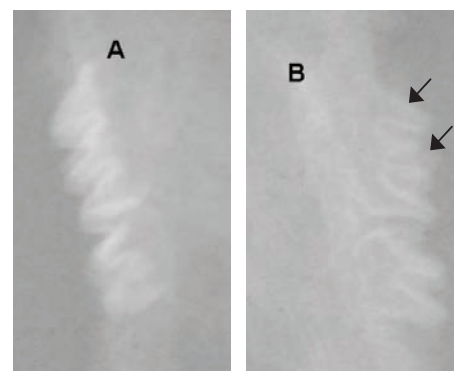
There was no evidence of hypoplasia (Figure 1A) on the rats of groups 1 with HHI index = 0 (either hypoplasia or hypocalcification undetected), meanwhile on the rats of group 2 hypoplasia was found in upper right molar (Figure 1-B). On radiopaque examinations showed that, group 1 had mean RGB higher than group 2, therefore, it could be suggested that the enamel showed more radiopaque companing to group 2 (Figure 2). The result of HHI examination and radiopaque feature of radiopaque could be seen on Table 1. Statistical analysis using T-test showed significant difference ( $p < 0,005$ ) between group 1 and 2 on HHI, radiopaque feature of radiopaque showed

insignificant difference ( $p > 0,005$ ) between group 1 and 2 (Table 2).



**Figure 1.** Rats molar enamel with and without enamel hypoplasia.

Note: A) Rat's molar in control group (without any treatment and enamel hypoplasia, B) Rat's molar in treated group, hypoplasia was found (LPS induction was done)



**Figure 2.** Radiopaque of rat's molar with and without enamel hypoplasia.

Note: A) Control group without treatment, B) Treated group with LPS induction. On figure B is more radiolucent compared to figure A → radiolucent enamel tooth

**Table 1.** The result of hypoplasia observation using hypoplasia hypocalcification index (HHI) and radiopaque observation using COREL DRAW version 11

N	Groups	HHI	radiopaque
1	I	0	196,6
2	I	0	198,4
3	I	0	195,73
4	I	0	196,2
5	I	0	198,2
6	II	8	180,5
7	II	8	88,4
8	II	8	170,87
9	II	1	179,6
10	II	3	102,1

Note: N = sample; HHI = hypoplasia hypocalcification index

**Table 2.** The result of T-test on the presence of hypoplasia and radiopaque score of rats tooth enamel

	t-test for Equality of Means						
	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
						Lower	Upper
HHI	-3.725	4.000	.020	-5.60000	1.50333	-9.77391	-1.42609
radiopak	2.608	4.006	.059	52.73200	20.21627	-3.36594	108.82994

Note: t= t count; df = degree of freedom; sig = significant; HHI = hypoplasia hypocalcification index

## DISCUSSION

The outcome of this study identified that LPS induction during the period of tooth growth and development resulting the presence of enamel hypoplasia. LPS consist of 3 parts: O-polysaccharide chain, core of polysaccharide and lipid-A. Lipid-A is a part of endotoxic LPS due to receptor CD14 bind / toll-like receptor-4 (TLR4).<sup>3</sup> CD14 is receptor of cell surface on macrophage and monocyte.<sup>16,17</sup> Macrophage and monocyte cell which make bacterial binding due to the presence of CD14 would secrete cytokine and lipid inflammation mediator.<sup>18</sup> The study done by Yoshimura *et al.*<sup>19</sup> suggested that LPS *A. actinomycetemcomitans* and *E. coli* are capable to induce PMN secreting IL-1 $\beta$ , IL-8, TNF- $\alpha$  and interleukin receptor antagonist (IL-1ra) in large number, while PMN which induced to LPS *P. gingivalis*, IL-1 $\beta$  is not detected. Monocyte which induced by LPS *P. gingivalis* and *E. coli* would secrete higher IL-1 $\beta$  than PMN. IL-1 $\beta$ , IL-8 and TNF- $\alpha$  stimulate the elevation of osteoclast amount and activities. Osteoclast is kind of cell which functions in degradation of bone matrix.<sup>6</sup> Induction LPS *E. coli* contributes the elevation of osteoclast number and activities and alveolar bone resorption.<sup>13</sup>

Either chronic or acute local infection in periodontal tissue which are presented during the period of tooth growth might contribute the failure of dental germ to perform the stage growth i.e. initiation (bud stage) proliferation (cup stage), histodifferentiation and morphodifferentiation (bell stage), apposition as well as calcification resulting the presence of abnormality of the form, number, quality and the quantity of eruptive teeth.<sup>20</sup> In enamel hypoplasia, failure is usually presented during the process of amelogenesis.<sup>2</sup> According to Mc Donald & Avery<sup>2</sup> and Wikipedia:<sup>20</sup> amelogenesis (enamel formation) is apart of the whole process of tooth growth. Amelogenesis occurs after perfect dentin formation done by ameloblast. Amelogenesis consist of two stages i.e. secretory stage (apposition) involving organic matrix formation and it is called enamel matrix, this stage functions during the process of enamel mineralization. The second stage is maturation i.e. enamel matrix has perfect calcification.<sup>2,20</sup> Infection which is presented during the period of enamel matrix formation would contribute the occurrence of hypoplasia

and if happens during the period of calcification would contribute enamel hypocalcification.<sup>2</sup>

The stage of matrix apposition and deposition done by formative cell would be the most sensitive stage during the period of growth.<sup>21</sup> LPS Induction resulting stimulation of osteoclast cell which could perform tissue resorption. Enamel prisms which has been deposited during apposition and deposition stage would be destroyed.<sup>22</sup> This case is due to stimulated osteoclast by LPS activity resulting the presence of resorption. In addition, ameloblast cell is the most sensitive cell environmental change.<sup>23</sup> Physiologic change would affect ameloblast and contribute enamel structural change. In general the change could not be seen clinically but it would appear through the microscope. Disturbance or infection might result the disturbance of enamel matrix secretion by ameloblast or death of ameloblast. The presented abnormality usually would appear clinically.<sup>1</sup>

Basically, the growth and the development of rats and human molar indicate the same stage during embryonal to eruption stage.<sup>24</sup> Molar amelogenesis on rats has initiated since the age of 20–21 days intrauterine and perfectly formed at the age of 11 days post uterine.<sup>21</sup> LPS induction done on 5 day-old rats resulting the disturbance of amelogenesis or going process. LPS contributes the presence of elevation IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, tumor necrosis factor - (TNF- $\alpha$ ) and PGE<sub>2</sub>, consequently, increasing the number and the activity of osteoclast. This case would cause obstruction of alveolar bone and the body would fail to stimulate the formation of fibrous wall which might localize the infection, as a result infection would disseminate on dental germ about 20–30%.<sup>25</sup> The number and the activity of osteoclast and odontoclast would continually degrade either base matrix or enamel matrix which is newly formed dental germ and disturb ameloblast cell. On 5 day-old rats ameloblast cell is forming enamel matrix and deposition process of organic and inorganic material is occurring. LPS induction would cause the death of ameloblast cell therefore enamel matrix with would fail to be formed. In normal condition ameloblast cell secretes enamel matrix with certain immunity.<sup>1</sup> Due to failure of enamel matrix formation, so there is some enamel which failed to be formed until the period of tooth eruption. As a result enamel becomes thin and either some holes would appear on the surface.

After the thickness of enamel matrix is considered adequate by ameloblast cell, the function of ameloblast cell would change and have important role in the process of enamel maturation. During the occurrence of maturation process, the change of enamel organic component is presented either qualitatively or quantitatively. Other changes also happen in enamel organic component i.e. calcium and phosphate influx presented in short time. This condition can cause the presence of crystal growth located in loophole which formed due to disappearance of organic material and water. Striated border and alkaline phosphates in ameloblast have importance role on organic ion transport through cell membrane enamel maturation.<sup>1</sup> The decrease and the presence of change on ameloblast cell due to LPS induction deposition process of either organic and inorganic material would be disturbed, so, the process of enamel mineralization is not perfect. Thin enamels due to mineralization disturbance which make the teeth look brownish yellow.

Brownish yellow color due to dentin appearance look transparent and several part of dentin opened. Dentin was looked yellower because the organic content is lower comparing to enamel. The radiopaque shows that rats with hypoplasia, the enamel is more radiolucent compared with control group (Figure 2). Solid material would adsorb X-ray stronger which causes the radiopaque clear (white) which is called radiopaque, while material with low density, the adsorption would be also weak, which gives dark region or it is called radiolucent.<sup>26,27</sup> In normal condition enamel would be more radiograph compared with other body tissue, because enamel consist of 90% mineral so enamel would be more solid.<sup>26</sup> On enamel hypoplasia due to the disturbance in matrix deposition either organic or inorganic the density of enamel is lower, as it is reported by Mahoney *et al.*<sup>28</sup> that tooth with hypoplasia would get lowering density 10% or between: 2,30–2,50 gr/cm<sup>3</sup>. The conclusion is LPS induction during tooth growth and development causing enamel hypoplasia.

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