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UNIVERSITAS SUMATERA UTARA
MEDAN

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Abstract

Systemic disorders have been implicated as a risk factor in periodontal disease. Conversely, severe and overwhelming periodontal disease may also play a role in the development of certain systemic diseases or adversely affect the control of systemic disease. Most systemic diseases manifest in the oral cavity, one of them is blood disorders (leukemia). Leukemia is a malignant disease characterized by proliferation of white blood cell-forming tissues and the abnormal increase of leukocytes in the blood circulation. The clinical features of patients with leukemia are in the form of gingival enlargement, hemorrhage, and mucosal ulceration. Knowledge about etio-pathogenic of periodontal issues in patients with leukemia is important for clinicians to detect early systemic abnormalities so that initial systemic therapy can be performed. Therapy procedure: Referring patient with leukemia to a specialist in internal medicine to resolve the systemic condition. After the systemic improved condition, then can be performed periodontal treatment. Conclusion: A dentist needs to know the symptoms and the clinical features of the oral cavity of leukemia patients to detect early the abnormalities in order to carry out systemic therapy. Management of oral and periodontal needs close team work between a dentist and a doctor.

Keywords: stain periodontal, Leukemia, Therapy

PENDAHULUAN

Penyakit keliman sistemik telah diklasifikasikan sebagai faktor risiko pada penyakit periodontal¹. Sebaliknya, penyakit periodontal yang parah dan tidak terkontrol juga dapat berperan dalam perkembangan penyakit sistemik tertentu atau berpengaruh buruk terhadap pengendalian penyakit sistemik tertentu². Kebanyakan penyakit sistemik bermanifestasi di dalam rongga mulut, salah satunya adalah kelainan darah (Leukemia).

Leukemia merupakan suatu keganasan dari sel darah putih dengan karakteristik proliferasi yang tidak terkontrol yang berasal dari sel-sel progenitor myeloid, jumlah dan bentuk abnormal dari leukosit yang belum matang pada periferis darah, serta penyebaran infiltrat pada hati, limpa, kelenjar limfe, dan bagian tubuh lain³. Leukemia

***Porphyromonas gingivalis* BACTEREMIA INDUCES INTRAUTERINE GROWTH RESTRICTION IN PREGNANT RATS**

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Abstract

During periodontal infection, when the oral mucosa is injured and inflamed, and the quantities of periodontal pathogens increase dramatically, transient bacteremia may occur. This can lead to selective colonization of undesired sites. The aim of this study was to examine the potential effect of *Porphyromonas gingivalis* bacteremia to intrauterine growth restriction in pregnant rats. Female rats were challenged with live *P. gingivalis* at concentration of 10^9 colony forming unit/ml into subgingival sulcus before and/or during pregnancy. This study was consisted of 4 groups i.e. group I, no *P. gingivalis* infection; group II, *P. gingivalis* infection before and during pregnancy; group III, *P. gingivalis* infection before pregnancy; and group IV, *P. gingivalis* infection during pregnancy. They were sacrificed on gestational day 20. Fetuses were evaluated for weight. *P. gingivalis* was detected by API-ZYM system in the maternal blood of the retro-orbital venous plexus and the umbilical cord. The percentages of IUGR at the time of sacrifice were 6.66% growth-restricted fetuses in group I, and 100%, 72.97% and 87.09% growth-restricted fetuses in group II, III, and IV, respectively. When weights of growth-restricted fetuses of the treated groups were compared to the control, there were significant differences ($P < 0.05$). *P. gingivalis* was first detected in the maternal blood, and the bacteria finally spread to the umbilical cord. This study represents that *P. gingivalis* may be transmitted hematogenously to the umbilical cord, and cause IUGR. The results strengthen the link between periodontal disease and adverse pregnancy outcomes.

Key words: *Porphyromonas gingivalis*; periodontitis; bacteremia; pregnancy; intrauterine growth restriction

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INTRODUCTION

Epidemiological studies have linked intrauterine growth restriction (IUGR) and the increased risk of developing preterm low birth weight (PLBW) babies. Although 25% to 50% of PLBW deliveries occur without any known etiology, there is increasing evidence that infection may play a significant role in preterm delivery¹. Several inflammatory disease are associated with reduced fetal growth, including rheumatoid arthritis², and periodontal disease^{3,4}. In addition, elevated maternal serum⁵ or placental inflammatory cytokines have been associated with IUGR⁶. Women with active inflammatory arthritis during pregnancy had smaller neonates at birth compared with health control women or women whose disease was in remission⁷, suggesting that active inflammation during pregnancy may contribute to a reduction in fetal growth.

An animal model is needed in order to investigate the association between local infection and fetal growth. Laboratory rats can be a useful model to study the mechanisms of human abnormal pregnancy outcomes⁸⁻¹⁰. This model of localized chronic infection with *Porphyromonas gingivalis* is adapted from Offenbacher and coworkers that used a mouse subcutaneous chamber model to study the effect of *P. gingivalis* infection on pregnancy outcomes in hamster¹¹ and mice¹² with heat-killed *P. gingivalis* induced a primary immune response. In addition, Han and coworkers injected *Fusobacterium nucleatum* into the tail vein of pregnant mice to study the ability of *F. nucleatum* to induce preterm birth or other adverse pregnancy outcomes when introduced into bloodstream of pregnant mice¹³.

In the present study, a rat chronic infection model will be challenged with live *P. gingivalis* into subgingival sulcus. This model more closely mimics the chronic infection with periodontal pathogen observed in human patients. Furthermore, will be tested the ability of *P. gingivalis* to induce IUGR when introduced into the bloodstream of pregnant rats. The aim of this study was, therefore, to examine the potential effect of *P. gingivalis* bacteremia to intrauterine growth restriction in pregnant rats.

MATERIALS AND METHODS

Animals and treatments. Adult female Sprague-Dawley rats weighed 150-200 g were used in this study, and maintained under controlled and standardized conditions. Rats were housed in conditions of 12-hour light-dark cycles from 7 a.m. to 7 p.m., and a

temperature of 25 °C. Regular rat diet and water were provided *ad libitum*. Rats were injected with 0.05 ml of 2×10^9 CFU/ml live *P. gingivalis* ATCC 33277 into the maxillary buccal and palatal gingival between first and second upper molars. The injections were repeated every other day on 3 separate days for 30 days and continued until 20 days after mating. The control group rats received 0.05 ml of PBS injection according to the same schedule as the *P. gingivalis*-injected rats. This study was consisted of 4 groups i.e. group I, no *P. gingivalis* infection; group II, *P. gingivalis* infection before and during pregnancy; group III, *P. gingivalis* infection before pregnancy; and group IV, *P. gingivalis* infection during pregnancy. The protocols and procedures were in accordance with the animal welfare guidelines and approved by The Institutional Animal Care and Use Committee, Universitas Gadjah Mada.

Timed mating. At least 4 weeks after induction of experimental periodontitis, female rats were mated overnight with male rats of the same strain. The next morning, females were removed from the male cages and examined for vaginal plugs. If a plug was found, that day was recorded as gestational day (GD) 1.

Sample collection. The pregnant rats were sacrificed on GD 20. Fetuses were removed post-mortem from the uterus and surrounding membranes. Each fetus was removed from its chorioamniotic sac and weighed to the nearest microgram. The resorption site and viable fetuses were counted and recorded for each rat. The viability of each fetus was assessed visibly. Fetuses were evaluated for weight and crown-tail length. IUGR was defined as fetuses with weight 2 standard deviations (SD) smaller than normal fetal weight (NFW; 3.56 ± 0.19 g)^{12,14}. Blood of umbilical cord was collected from each fetus and pooled per dam. Maternal blood was bled from the retro-orbital venous plexus, and collected. All samples were stored at -80°C until analysis.

Detection of *P. gingivalis* bacteremia. Maternal blood of retro-orbital venous plexus and blood of umbilical cord was immediately plated on tryptic soy agar containing sheep blood and grown for 5-7 days at 37°C under anaerobic conditions. *P. gingivalis* colonies were identified by their black pigment, Gram staining and API-ZYM system, and then were compared to *P. gingivalis* ATCC 33277 for confirmation of organism. The API-ZYM colorimetric kit system (bioMérieux SA, Marcy-l'Etoile, France) for detection of enzymes was used according to the direction of the manufacturer. Color reactions were read with a grade scale in which 0 indicated no

enzyme activity, 1 or 2 indicated weak activity, and 3 to 5 indicated strong enzyme activity. Key differential tests for oral species of *Bacteroides* described that *P. gingivalis* were very consistent and distinctive for trypsin-like activity, uniformly negative for α -Glucosidase and *N*-Acetyl- β -glucosamidase¹⁵.

Statistical analysis. The fetal weights were presented as mean values \pm the SD of the mean. Statistical analysis of compared mean fetal weights between groups were performed using one-way analysis of variance. The mean difference was significant at the <0.05 level.

RESULTS

Periodontal infection were found in the challenged animals. During the course of experiment, the challenged animals had no febrile, did not exhibit malaise, and did not lose weight as a consequence of challenge. When comparing the mean fetal weights from dams challenged with *P. gingivalis* (group II, III, and IV) to the control dams, they significantly resulted in a decrease in the mean fetal weights than the control group ($P<0.05$). The percentages of IUGR at the time of sacrifice GD 20 are also shown in Table 1. There were 6.66%, 100%, 72.97% and 87.09% growth-restricted fetuses in group I, II, III, and IV, respectively. When weights of growth-restricted fetuses of the treated groups were compared to the control, there were significant differences ($P<0.05$).

Tabel 1. Pregnancy outcomes in *P. gingivalis*-infected pregnant rats observed at GD 20

Groups	Mean fetal weight \pm SD (g)	IUGR/Total fetuses (%)
I	4.079 \pm 0.430	2/30 (6.66%)
II	0.565 \pm 0.168*	43/43 (100%)
III	2.729 \pm 0.500*	27/37 (72.97%)
IV	2.342 \pm 0.582*	27/31 (87.09%)

*Significantly lower than control group ($P<0.05$)

In the control group, all maternal blood of retro-orbital venous plexus and umbilical cord samples from normal-weighted fetuses and growth-restricted fetus were not detected *P. gingivalis*. Whereas, maternal blood of retro-orbital venous plexus and umbilical cord samples from the treated groups possessed variable results of enzymatic activities of *P. gingivalis* as measured by the API-ZYM system. In the treated groups at GD 20 showed a strong of trypsin-like activities, and uniformly negative for α -Glucosidase and *N*-Acetyl- β -glucosamidase (Table 2 and 3).

Table 2. Average fetal weight and presence of *P. gingivalis* in maternal blood of retro-orbital venous plexus and umbilical cord from fetuses among *P. gingivalis*-infected dams observed at GD 20

Groups	Presence of <i>P. gingivalis</i>	Number of fetuses	Mean fetal weight
			\pm SD (g)
I	Yes	0	0
	No	30	4.079 \pm 0.430
II	Yes	22	0.499 \pm 0.157
	No	21	0.634 \pm 0.154
III	Yes	12	2.380 \pm 0.168
	No	25	2.898 \pm 0.522
IV	Yes	14	2.107 \pm 0.571
	No	17	2.535 \pm 0.531

Table 3. Enzymatic activities of *P. gingivalis* from blood of retro-orbital venous plexus and umbilical cord observed at GD 20

Groups	Test result								
	Trypsin			α -Glucosidase			<i>N</i> -Acetyl- β -glucosamidase		
	Neg.	Weak	Strong	Neg.	Weak	Strong	Neg.	Weak	Strong
I	18	12	0	6	6	18	6	12	6
II	15	4	22	22	11	8	24	9	8
III	14	11	12	16	14	7	14	11	12
IV	7	10	14	23	5	3	18	7	6

Neg.: negative

DISCUSSIONS

During periodontal infection, when the oral mucosa is injured and inflamed, and the quantities of periodontal pathogens increase dramatically, transient bacteremia may occur¹⁶. This can lead to selective colonization of undesired sites. In the current study, we proposed initial transmission of organisms from oral cavity into bloodstream and addressed the question of what effects of *P. gingivalis* has on pregnancy if it enters the circulation.

Porphyromonas gingivalis reaches high proportions in plaques associated with advanced periodontitis but are rarely detected in health. The trypsin-like activity seems to be unique to this bacteria as more than 25 other oral species are known¹⁷. The presence of this trypsin activity primarily in periodontopathic organisms suggests that this enzyme may be an important determinant of their virulence in periodontal disease. Such proteolytic activity may have a direct effect upon the junctional epithelium in the periodontal pocket as trypsin has been shown in vitro to disrupt cell-cell or cell-substratum adhesions¹⁸. In addition, trypsin seems to activate latent gingival tissue collagenase by destruction of a collagenase inhibitor present in serum¹⁹. Finally, this bacterial enzyme could activate the alternate pathway of complement fixation causing the release of leukotactic factors C3a and C5a, as has been demonstrated with trypsin and certain bacterial proteinases²⁰. In fact, this trypsin-like enzyme could be the factor in periodontal plaque²¹ and pure cultures of *P. gingivalis*²² which has been found to be chemotactic for polymorphonuclear leukocytes. These trypsin-like activities acting singly or in concert could effect significant pathology on the periodontium.

Porphyromonas gingivalis also have been shown to resist phagocytosis even in the presence of specific antibodies and factor C3 of the complement system²³. Recently, Sundqvist²⁴ showed that *P. gingivalis* W83 was able to degrade complement proteins C3 and C5 from guinea pig serum both in vitro and in vivo. By degrading complement and immunoglobulins, *P. gingivalis* may evade the phagocytic host defense.

Blood was sampled immediately following *P. gingivalis* injection in subgingival sulcus. Animal studies have shown that peak bacteremia occurs quickly within the first minute when human oral microorganisms are injected into the bloodstream²⁵, and systemic dissemination has been reported within 40 minutes of commencing dental procedures²⁶. The timing may be an important factor in determining the amount of

bacteria recovered from the bloodstream and whether a bacteremia can be detected at all. The reduction bacteremia over several minutes after dental instrumentation is due to the effectiveness of host defence system in rapidly clearing microorganism from the blood²⁷. In our study, the bacteria were usually cleared within 10 minutes and always within 20 minutes.

Our study showed that maternal *P. gingivalis* infection on periodontal tissue can result in *P. gingivalis* dissemination to umbilical cord and induction of IUGR, but *P. gingivalis* was not always detected in the umbilical cord from abnormal pregnancies. Several possibilities could explain why *P. gingivalis* was detected in some affected dams but not others. It might be attributed to the technical aspects of the culture technique, mainly the usage of non-specific medium to grow the microorganisms. Alternatively, the effect of *P. gingivalis* on IUGR may be mediated by bacterial products or by host mediators, rather than direct dissemination in some *P. gingivalis*-infected dams.

In general, there were significantly more IUGR fetuses in the *P. gingivalis*-infected groups than in the control, and difference percentage of IUGR fetuses were observed between the *P. gingivalis*-infected groups. A fetus can be smaller in size due to a general retardation in overall development compared to its littermates, or it can be developmentally normal but lack in weight gain.

The role of bacterial infections in pregnancy complications is well known. Bacterial vaginosis and chorioamnionitis can lead to spontaneous preterm birth, especially in early gestation^{28,29}. Studies showed that intrauterine infection were common among women who gave birth prematurely^{30,31}. Four possible mechanism exist for microbes to spread to the uterus, which otherwise is a sterile environment: 1) organisms from vagina and the cervix ascend to the uterus; 2) the organisms originate elsewhere in the body and infect placental tissues as a result of hematogenous spread; 3) organisms from the peritoneal cavity translocate retrogradely through the fallopian tube; and 4) organisms are inoculated accidentally in uterine tissues during invasive procedures, such as amniocentesis or chorionic villous sampling³¹. Hence, the putative link between periodontal disease and pregnancy complications might be attributable to repeated exposures of the decidual tissues to periodontal pathogens through transient bacteremia.

In humans, oral microorganisms, including *Fusobacterium nucleatum* and *Capnocytophaga sputigena*, were detected in the amniotic fluid of women with intact membranes³²⁻³⁴ and those with preterm labor³⁵, thus supporting the possibility that oral bacteria or bacteria products can spread through the bloodstream to the placenta. Oral pathogens presumably gain access to the systemic circulation via local tissue inflammation and breakdown, as demonstrated in animal models, and might cause damage by affecting the placenta and possibly the fetus itself.

The evidence of *P. gingivalis* in blood of umbilical cord of dams with IUGR may support a possible role of this microorganism in the pathogenesis of IUGR, making maternal oral infectious exposure a crucial phenomenon throughout pregnancy.

Once our present findings are established further and corroborated, future research will be focused on preventive strategies aimed at reducing oral bacterial load, which hopefully would decrease the incidence of IUGR.

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