

## Plasma Fibronectin in Saliva of Women with History of Miscarriage(s) and Periodontal Disease - a Pilot Study

(Fibronektin Plasma di dalam Air Liur Wanita Mengandung dengan Sejarah Keguguran dan Penyakit Periodontitis Kronik- Kajian Permulaan)

FARHA ARIFFIN\*, SALMAH KAMIN, JAMIYAH HASSAN & HALIZA HASAN

### ABSTRACT

*The objective of this study was to establish the association of plasma fibronectin in saliva of pregnant women with history of miscarriage(s). A total of 25 pregnant women with history of miscarriage(s) were recruited at the Antenatal Clinic of University Malaya Medical Centre (UMMC), Kuala Lumpur as the test group and 16 pregnant women without any history of miscarriage served as control. All the subjects fulfilled a set of inclusion and exclusion criteria. Periodontal examination was performed to determine subjects' periodontal status. Saliva samples were collected and enzyme-linked immunosorbent assay (ELISA) was used to detect the plasma fibronectin in these samples. Mann-Whitney test was used to determine the significance of differences seen. The study showed a statistically significant higher median plasma fibronectin level in the saliva samples of the test group with a median level of 0.10 µg/mL compared with the control group with a mean value of 0.00 µg/mL (p-value = 0.023). However, there was no significant difference in the level of plasma fibronectin in the saliva samples of pregnant women with chronic periodontitis compared with the group with healthy gingiva (p-value = 0.118). As a conclusion, there was a positive association between plasma fibronectin in saliva of women and history of miscarriages compared with the control group in this case study.*

*Keywords: Miscarriage; periodontal disease; plasma fibronectin*

### ABSTRAK

*Objektif kajian ini adalah untuk melihat hubung kait antara fibronektin plasma di dalam air liur ibu mengandung dengan sejarah keguguran. Seramai 25 ibu mengandung dengan sejarah keguguran di Klinik Ibu Mengandung Pusat Perubatan Universiti Malaya, Kuala Lumpur telah dikenal pasti sebagai kumpulan kajian manakala seramai 16 lagi ibu mengandung tanpa riwayat keguguran dijadikan kumpulan kawalan. Kesemua subjek kajian menepati kriteria yang telah ditetapkan. Pemeriksaan periodontium telah dilakukan bagi mengenal pasti status kesihatan periodontium mereka. Sampel saliva mereka dianalisis menggunakan ujian 'enzyme-linked immunosorbent assay' (ELISA) untuk mengesan kandungan fibronektin plasma. Ujian Mann-Whitney digunakan bagi menganalisis data. Keputusan mendapati aras purata fibronektin plasma di dalam kumpulan kajian adalah melebihi berbanding purata fibronektin plasma dalam kumpulan kawalan. Walau bagaimanapun, tiada perbezaan ketara yang ditemui bagi purata fibronektin plasma antara kumpulan subjek yang mempunyai penyakit periodontitis kronik berbanding subjek yang mempunyai periodontium yang sihat. Kesimpulannya, kajian ini menunjukkan bahawa terdapat hubung kait yang positif antara aras fibronektin plasma di dalam saliva subjek dengan riwayat keguguran.*

*Kata kunci: Air liur; fibronektin plasma; keguguran; penyakit periodontium*

### INTRODUCTION

History of miscarriages has an impact on the outcome of future pregnancy. It has been established as one of the significant risk factors for adverse pregnancy outcomes including recurrent miscarriage (Regan et al. 1989) and preterm labour (Mercer et al. 1999). A recent study showed that a woman who has had miscarriage in their initial pregnancy was at a higher risk of developing obstetric complications such as pre-eclampsia, preterm labour and low birth weight babies (Bhattacharya et al. 2008).

The knowledge of risk factors is important in recognizing an individual's susceptibility to adverse pregnancy outcomes. Traditionally, the patients at risk

can be identified by taking a thorough medical and obstetrical history. However, history alone has low sensitivity. Researches in these areas are being focussed at identifying host susceptibility using various host markers to aid in the prediction of adverse pregnancy outcomes. One of the important markers that had been widely used to predict preterm labour was foetal fibronectin from the vaginal swabs of pregnant women (Leitch & Kaider 2003; Lockwood et al. 1991; Shennan et al. 2005).

Fibronectin is a glycoprotein present in blood, connective tissue matrix and on cell surfaces. It can exist in the body fluids as a soluble form or as an insoluble form in the connective tissue matrix protein (Ruoslahti 1981). It

acts as a component for cellular adhesion to surrounding matrix as fibronectin possesses specific binding sites for collagen (Kleinman et al. 1978), fibrinogen (Ruoslahti & Vaheiri 1975) and heparin (Stathakis & Mosesson 1977). Fibronectin was also incorporated into blood clots (Ruoslahti & Vaheiri 1975) and was assumed to have the ability to bind to fibrin by providing adhesive matrix for cells to grow in the blood clot (Ruoslahti 1981).

Fibronectin had been used in medical research as a marker for the presence of sepsis (Ruiz Martin et al. 2004) to monitor the severity of hepatic inflammation (Kandemir et al. 2004) and to detect cancer (Menendez et al. 2005). Similarly, the presence of plasma fibronectin in saliva samples may be an indicative of systemic disease and thus it could be used as a host marker for certain pathological condition as well as being a marker for local tissue destruction as in periodontal disease (Babu et al. 1983; Lopatin et al. 1989).

Fibronectin was also found in stimulated whole saliva (Babu et al. 1983). However, it was not known whether the fibronectin originated from salivary glands or from gingival exudates. Since gingival exudates contains several plasma proteins, plasma fibronectin was assumed to be one of the component in the gingival crevicular fluid since fibronectin was then detected in the samples of whole saliva, gingival crevicular fluid and gland-specific saliva excluding parotid gland (Tynellius-Brathall et al. 1986). From the study, they found that the amount of fibronectin per  $\mu\text{g}$  protein did not differ significantly between plasma and gingival crevicular fluid. The association of fibronectin with pathogenesis of periodontal disease and whether it gave an impact on healing of periodontal structures or not, was inconclusive because there were no statistically significant differences in concentrations of fibronectin in human saliva between pre and post-periodontal treatment (Tynellius-Brathall 1988).

The aims of the study were to determine the association of plasma fibronectin in the saliva of pregnant women with a history of miscarriages and to determine the association of plasma fibronectin in saliva with periodontal disease.

#### MATERIALS AND METHODS

This is a case-control study which involved 41 consecutive pregnant women with past history of miscarriage in their second trimester up to 32 weeks of gestation who came for antenatal check-up in Antenatal Clinic, University Malaya Medical Centre (UMMC) Kuala Lumpur. The study was carried out from October 2007 until June 2008. Pregnant women who were smokers, had history of drug abuses, had multiple foetuses and presence of serious medical or systemic conditions were excluded from the study. All subjects aged between 18 and 42 years old and had consented for the study were then asked to undergo periodontal examination at the Periodontology Postgraduate. Their periodontal status was recorded. Periodontal disease in the form of chronic periodontitis was diagnosed based on the presence of these criteria: Probing pocket depth of  $\geq 5$  mm at  $\geq 4$  sites for the whole mouth

and bleeding on probing at these sites. In addition, the information on their history of miscarriage(s) was obtained from the medical records. The study was conducted with the approval from the Medical Ethics Committee of University Malaya Medical Centre (No 607.1).

Each subject was asked to expectorate into a sterile plastic tube and a 4 mL unstimulated whole saliva was collected which was then frozen at  $-80^{\circ}\text{C}$  for analysis. The collection of the saliva samples was standardized before 12.00 pm to avoid differences in the content caused by circadian rhythm and done prior to periodontal examination to avoid blood contamination to the samples.

The level of salivary fibronectin in each samples were analysed using a commercially-available Enzyme-linked immunosorbent assay (ELISA) kit (IMUCLONE<sup>®</sup>) of murine monoclonal antibody to human fibronectin.

Statistical Package for Social Science (SPSS) Version 16.0 was used to analyze the results. Since the fibronectin level in the saliva displayed a non-normal distribution, the Mann-Whitney test was used to determine the significance of the difference between the test and control groups. The statistical significance was set for a  $p$ -value of  $\leq 0.05$ .

## RESULTS

#### DEMOGRAPHIC PROFILES OF THE SUBJECTS

The sample consisted of 25 consecutive healthy pregnant women (Group A) who came for antenatal check-up and had history of previous miscarriage(s) which occurred between the 6<sup>th</sup> and the 27<sup>th</sup> week and subsequent 16 healthy pregnant women (Group B) who had no previous miscarriage and who has had deliveries at more than 36 weeks. Fourteen women in Group A had one miscarriage and the other eleven of them have experienced twice. None of the subjects have experienced more than twice miscarriage(s) (Table 1).

All 41 subjects examined were included in this case study. Majority of the subjects were Malays with 33 (54.9%), followed by Indians 7 (23.4%) and Chinese 1 (20.0%) as presented in Table 2. The average (mean and median) levels of fibronectin were higher among subjects in Group A and C as compared with subjects in Group B and D.

#### PLASMA FIBRONECTIN IN TEST AND CONTROL GROUPS

Using Mann-Whitney test, our results showed that there was a significant difference in the level of plasma fibronectin in the saliva of group A and B (Table 3 and Figure 1).

The Group A subjects were further analyzed using Mann-Whitney Test. The result showed that the difference in fibronectin level was not significant between those diagnosed with periodontal disease and healthy subjects. The median gap was rather small. However, for Group B subjects, there was a significant difference in fibronectin level between periodontal disease and healthy subjects.

The median gap was larger for these two groups (Table 4). Combining the two groups did not produce any significant difference between subjects with periodontal disease and healthy periodontium in the value of fibronectin with the  $p$ -value 0.118.

#### DISCUSSIONS

Traditional teaching uses medical or obstetric history to identify women at risk of miscarriage. Advanced maternal age has also been to play a role. Researches on definitive markers to identify women at risk of miscarriages are too few. A host response marker would be useful to enable us to recognize an individual who is susceptible to miscarriage. Unfortunately, markers for miscarriage have not been as well-established as compared with those currently used in obstetrics for preterm labour.

Apart from miscarriage, the increased levels of plasma fibronectin have been associated with other adverse pregnancy outcomes such as pre-eclampsia (Dreyfus et al. 1998; Friedman et al. 1994; Stubbs et al. 1984) and

pre-term labour (Zygmunt et al. 1997). The level of plasma fibronectin is also higher in maternal plasma samples of patients with preterm delivery as compared with normal pregnant women (Zygmunt et al. 1997). The results from this study illustrates that the fibronectin level of pregnant women with past history of miscarriage(s) is statistically significant higher than in the group that never had a miscarriage.

Even though the amounts of fibronectin detected was low in both test and control groups, it is generally accepted that the fibronectin levels in saliva and GCF are lower than that of the plasma (Lopatin et al. 1989), which is usually at 1% below the average plasma level. From this study, the origin of plasma fibronectin was probably due to a systemic condition. The mean value of plasma fibronectin in test subjects without periodontal disease was higher compared with control subjects without periodontal disease. This demonstrates that the prevailing high fibronectin level even in healthy subjects of test group was not entirely due to local tissue destruction. In group B, the high value of plasma fibronectin in those with periodontal disease

TABLE 1. Distribution of subjects according to groups

	Periodontal status		
	Periodontal disease	Healthy	Total
History of miscarriage(s)	Yes	11	14
	No	5	11
	Total	16 (Group C)	25 (Group D)
			25 (Group A)
			16 (Group B)
			41

TABLE 2. Demographic profiles of the subjects based on miscarriage(S) history and periodontal status

History of miscarriage	Periodontal status	N	Malay	Chinese	Indian	Mean Age	Mean fibronectin level	Median fibronectin level
Yes	Periodontal disease	11	7	1	3	29.55	0.42	0.15
	Healthy	14	11	0	3	29.07	0.37	0.09
	Total	25	18	1	6	29.28	0.39	0.10
No	Periodontal disease	5	4	0	1	30.40	0.25	0.18
	Healthy	11	11	0	0	30.82	0.01	0.00
	Total	16	15	0	1	30.69	0.09	0.00
Total	Periodontal disease	16	11	1	4	29.81	0.37	0.16
	Healthy	25	22	0	3	29.84	0.21	0.00
	Total	41	33	1	7	29.83	0.27	0.19

TABLE 3. Plasma fibronectin in saliva between group A and B

History of miscarriage	N	Median fibronectin level ( $\mu\text{g/mL}$ )	$p$ -value
Group A	25	0.1	0.0234
Group B	16	0	

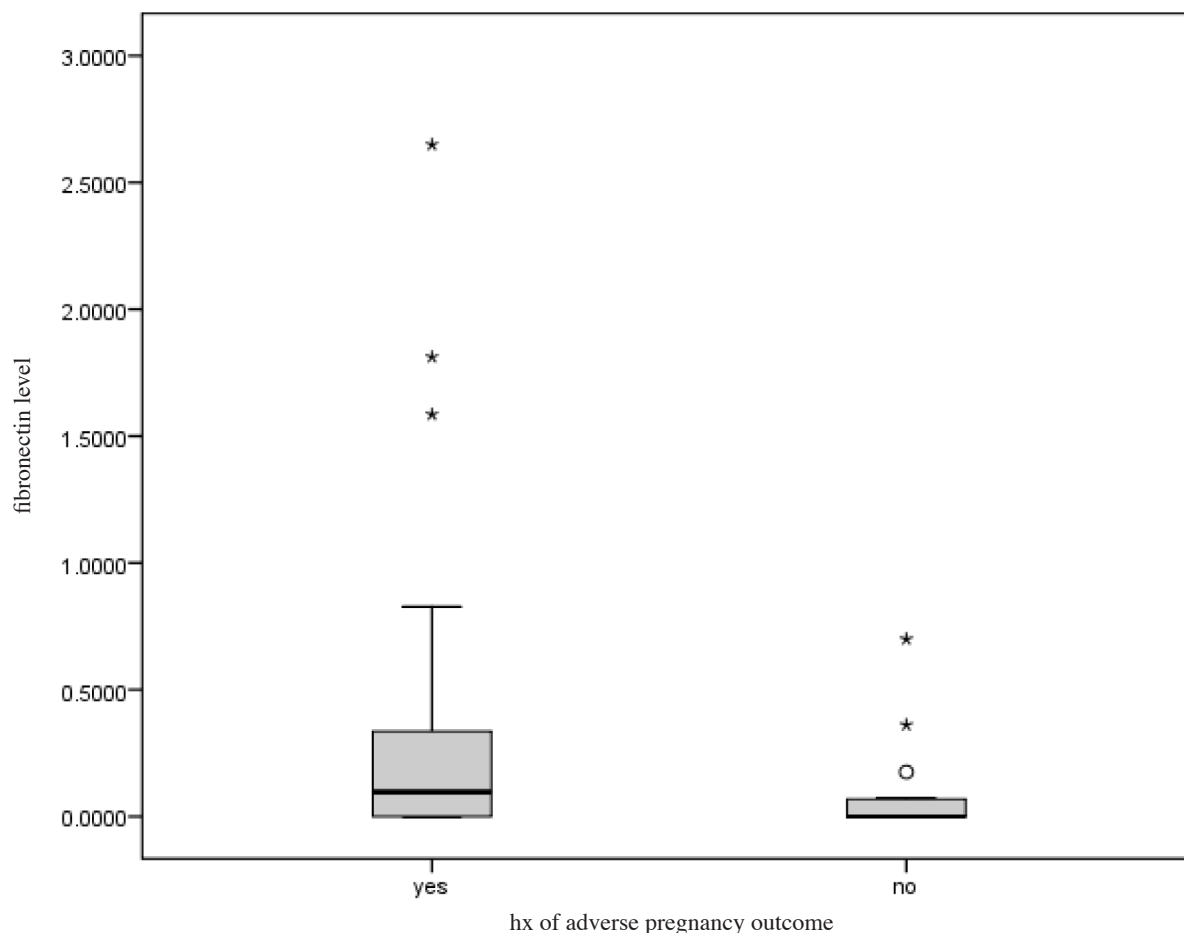


FIGURE 1. Plasma fibronectin in saliva between group A and B periodontal status

TABLE 4. Plasma fibronectin in saliva between groups in relation to periodontal status

History of miscarriage	Periodontal status	Median fibronectin level ( $\mu\text{g/mL}$ )	Median gap	<i>p</i> -value
Yes (Group A)	Periodontal disease	0.15		
	Healthy	0.09	0.06	0.889
No (Group B)	Periodontal disease	0.18		
	Healthy	0	0.18	0.046
Total	Periodontal disease	0.16		
	Healthy	0	0.16	0.118

was probably due to local tissue destruction as there was significant difference of fibronectin levels between the periodontal disease and healthy subjects. This was probably due to the fact that there is an increased permeability of the blood vessels in the periodontium during periodontal disease. This results in the increase in fibronectin exudation into the surrounding tissue, flushing into crevice region and ending up in the saliva.

Plasma fibronectin is synthesized by endothelial cells and forms a layer between vascular endothelium and the basement membrane (Mosesson & Armani 1980). The increase in plasma fibronectin can be due to endothelial

damage or alteration in endothelial function (Stubbs et al. 1984). This alteration in endothelial function caused thrombus formation and thrombotic abnormalities (Clark et al. 1999). Thrombus formation in blood vessels to maternal-foetal environment could lead to failure of foetal development as thrombotic events have been postulated as one of the possible mechanism in miscarriage (Laird et al. 2003).

Elevated levels of plasma fibronectin may not only be the result of an alteration or damage in endothelial cells, but it could also be the causative factor for the vascular disturbances, as it has also been associated with thrombotic

events. The interaction of plasma fibronectin with fibrin, collagen and activated platelets is important in a thrombotic event as evidenced by animal studies where the thrombus growth in injured arterioles is delayed with depletion of plasma fibronectin (Matuskova et al. 2006). Fibronectin is cross-linked to fibrin during thrombus formation and this further enhances the stability of the clot (Kamykowski et al. 1981).

Most clinicians agree that a significant number of women with miscarriage(s) have a haemostatic disturbance in the blood vessels to the foetal-maternal circulatory system (Younis 1998). This is because pregnancy itself is a hypercoagulable state and predisposes to thrombus formation and cause placental vascular insufficiency, leading eventually to foetal death (Younis 1998).

Our study has some limitations that should be considered for future study. First, saliva sample alone is not enough to study for the association of plasma fibronectin found in the saliva with miscarriage. Cervicovaginal swab is the other sample that should have been incorporated in the study. However, the procedure is only allowed for medical officers or midwives. It would be better if we could compare the level of fibronectin saliva and in cervicovaginal swab of each subjects in order to determine the correlation between those two sources of the fibronectin. We should also collect the blood samples to compare the fibronectin level in saliva and the plasma samples since more studies have established the significance relevant of plasma fibronectin in plasma samples.

Secondly, the diagnosis of periodontal status among pregnant women in this study is in very restricted situation where it was done mainly based on clinical examination and not with the aids of radiograph findings. Chronic periodontitis was diagnosed based on the pocket depth more than 4 mm in at least 4 sites of the dentition (Van der Velden 2000). The diagnosis should involved more than 4 sites because four sites is too minimal to show the generalized involvement of chronic periodontitis. In fact, the periodontal parameter should involved pocket depth more than 5 mm with attachment loss at least 4 mm (Lopatin et al. 1989).

Thirdly, the selected cohort may not be suitable since it involved only urban population that were generally more-educated and aware of their oral health conditions.

As a conclusion, there was a positive association between plasma fibronectin in saliva of women with history of miscarriage but no association was found between plasma fibronectin in saliva with periodontal disease in this case study. They had a higher level of plasma fibronectin compared with the control group. Thus, plasma fibronectin in saliva could be a potential marker for clinical diagnosis of miscarriage.

For future study, we would like to recommend for the involvement of a larger sample size from multiple centres. The study should be done together with representative from obstetrics and gynecology. Serum and cervicovaginal swab samples should also be acquired

from each subject together with the saliva. Fibronectin levels from the three sources should then be compared among pregnant women that had miscarriages. This could show the relevance of fibronectin levels from the saliva as a surrogate for the serum levels of the same, indicating systemic dissemination of fibronectin. Timing of data collection should include those in the early trimesters of pregnancy. These women should be followed up until the day of delivery or the miscarriage. Sampling should be done periodically to detect chronological changes in the fibronectin levels of all 3 sampling sites (saliva, serum and vagina). Finally, the recruitment of samples should ideally be those who have been diagnosed with chronic periodontitis prior to conceiving. This is to assure the accuracy of the diagnosis of periodontitis, as these patients would have had an intraoral radiograph done.

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Farha Ariffin\*  
Faculty of Dentistry  
Universiti Teknologi MARA  
40450 Shah Alam  
Selangor  
Malaysia

Salmah Kamin  
KPJ Selangor Specialist Hospital  
40300 Shah Alam  
Selangor  
Malaysia

Jamiyah Hassan  
Department of Obstetrics and Gynaecology,  
University Malaya Medical Centre  
50900 Kuala Lumpur  
Malaysia

Haliza Hasan  
Faculty of Computer Science and Mathematics  
Universiti Teknologi MARA  
40450 Shah Alam  
Selangor  
Malaysia

\*Corresponding author; email: drfarha@salam.uitm.edu.my

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