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ORIGINAL ARTICLE

Preventive hygiene protocol of University of Milan for women during pregnancy: A qualitative and quantitative bacterial plaque analysis prospective original study

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KEYWORDS

Oral health in pregnant;
Bacterial plaque;
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Abstract Introduction: The aim of this article is to describe the preventive hygiene protocol of University of Milan for women during pregnancy analyzing the bacterial plaque quantitatively and qualitatively.

Materials and methods: A sample of 35 pregnant women following a protocol of periodic visits starting from the first month of pregnancy until the childbirth and in follow up controls were analyzed. Several samples (n = 4) of bacterial plaque for quantitative and qualitative analysis were taken, from the lingual surface of the lower first molar, during the first visit (T0), during the first trimester (T1), during the second or third trimester of pregnancy (T2), and one month after childbirth (T3).

Results: By performing a quantitative analysis, it was calculated that the average plaque index (Fig. 1) was n = 48.1% (T0), n = 14.7% (T1), n = 18.4% (T2) and n = 18.9% (T3). The plaque index score presents a downward trend, passing from 48.1% (T0) to 18.9% (T3). The number of total cocci (Fig. 2) was n = 205.39 (T0), n = 57.5(T1), n = 74.6 (T2) and n = 75.4(T3). The number of total bacilli (Fig. 3) was n = 62.7 (T0), n = 23.1 (T1), n = 25.3 (T2), n = 27.1(T3). The total values of cocci and bacilli were correlated and the average trend of the various samples was calculated. By performing a qualitative analysis, the value of G+ cocci (Fig. 5) was n = 2.7 (T0), n = 1.4 (T1), n = 1.4 (T2) and n = 1.5 (T3). The value of G- cocci (Fig. 5) was n = 2.3 (T0), n = 0.7 (T1), n = 1.1 (T2) and n = 1.1 (T3). The value of G+ bacilli (Fig. 6) was n = 1.6 (T0), n = 0.9 (T1),

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$n = 1.2$ (T2) and $n = 1.2$ (T3). The value of G- bacilli (Fig. 6) was $n = 1.3$ (T0), $n = 0.3$ (T1), $n = 0.7$ (T2) and $n = 0.7$ (T3).

Conclusions: The preventive hygiene protocol used in the Dental Hygiene Department of the University of Milan, during the gestation period, is a suitable method for the control of the bacterial plaque. A considerably decrease of the plaque index and bacterial components between the first visit and the subsequent check-ups was calculated.

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1. Introduction

Pregnancy is a complex event that causes alterations throughout the mother's body. Notable systemic effects are those of the gastrointestinal system, renal function, cardiovascular apparatus, blood composition and coagulation, and respiratory function.

The woman's body adapts to the ongoing changes (Flynn, 2007).

At the metabolic level there is a total average weight increase due to the development of breasts, uterus, fetus and embryofetal appendages and as a result of water retention facilitated by the fall in plasma osmolarity. The woman's body adapts to the ongoing changes (Flynn, 2007). The increase in circulating levels of progesterone and estrogen leads to a decrease in gastrointestinal motility that results in a subjective feeling of hypersalivation, often associated with nausea. The esophageal function remains normal, even if the frequency of gastro-oesophageal reflux, often due to pyrosis, increases.

Gastric tone and motility decrease, while the production of gastrin and gastric volume increases with a decrease in pH. All these factors predispose the subject to nausea and vomiting. Several modifications occur in oral cavity causing alterations in salivary composition, onset of caries and periodontal disease, that represent a risk factor for unfavourable outcomes during pregnancy, such as prematurity and low birth weight, as well as the presence of specific types of bacteria, such as *P. gingivalis*, *T. forsythia*, *A. actinomycetemcomitans*, *P. intermedia* and *T. denticola* (Emmatty, 2013).

The importance of oral health care practices in pregnant women is important to reduce and to prevent pregnancy complications.

The aim of this article is to describe the preventive hygiene protocol of University of Milan for women during pregnancy analyzing the bacterial plaque quantitatively and qualitatively.

2. Materials and methods

2.1. Group of study

For this study, a sample of 35 pregnant women were enrolled in a protocol of periodic visits starting from the first month of pregnancy until the childbirth and in follow up.

Inclusion criteria were: good compliance and presence at all visits ($n = 4$) required during the study. All patients were informed at each stage of the study and they expressed their informed consent. The protocol included four steps, in each of them standardized and specific procedures were performed.

Exclusion criteria were: the presence of orthodontic devices that could alter the bacterial flora; smoking patients (Lucchese, 2018).

Four samples ($n = 4$) of bacterial plaque for quantitative and qualitative analysis were taken, from the lingual surface of the lower first molar, during the first visit (T0), during the first trimester (T1), during the second or third trimester of pregnancy (T2), and one month after childbirth (T3). The plaque index was detected with the method described by Silness and Løe (1964).

Ethical approval for this study: n°421, 09/03/2016, Fondazione Ca' Granda, Ospedale Maggiore Policlinico, Milano, Italia.

The study was designed in accordance to principles of the Declaration of Helsinki for medical research involving human subjects.

2.2. Level 1. First visit

During the following procedures were performed:

- medical history and clinical examination;
- plaque (P.I.) and gingival bleeding index (G.B.I.);
- psychological motivation;
- oral hygiene instruction.

In the early stages of pregnancy, the hygienist gave instructions to the patient about the most appropriate oral hygiene maneuvers.

During the first visit it was also requested to note in a food diary all the foods taken daily for the duration of one week. Salivary tests were performed to assess if the subject is at risk of caries.

In pregnant women with a high rate of Streptococcus mutans, a treatment with fluorine and chlorhexidine was scheduled.

2.3. Level 2. Procedures in the first trimester of pregnancy

In the first trimester, it was important to provide the patient with detailed oral hygiene instructions, periodically scaling and polishing, help with the compilation of a food diary that should be checked periodically in order to make corrections and changes.

The patient must be informed about plaque control in order to avoid the onset of caries and periodontal disease in relation to the gestational state.

When the patient is motivated by the conscientious practice of oral hygiene procedures, associated with professional prophylaxis, periodontal disease should not occur.

Proper professional and home hygiene has proved to be decisive for a reduction in local phlogistic processes and advantageous for the reduction of the microflora present at the gingival level (Lucchese, 2018).

The role of the hygienist is to eliminate all possible sources of chronic irritation such as plaque or tartar, to help the patient in the maneuvers of oral hygiene, both with professional hygiene sessions and in the choice of the most suitable means of home hygiene.

2.4. Level 3. Procedures in the second and third trimester of pregnancy

In the second and especially in the third trimester, it was necessary to reinforce motivation, underlining that regular oral hygiene avoids gingivitis and periodontal disease. Furthermore it was important to pay attention to the diet and schedule check-up appointment.

Only if the patient has gained the conviction that oral hygiene leads to a benefit, she performs it regularly. The motivation must be renewed through periodic check-ups, in which a new sample of plaque is collected for quantitative and qualitative analysis. Control sessions and motivation have been scheduled every two months. A special oral hygiene protocol has been applied to pregnant women subject to frequent acid regurgitation, in which it was suggested to rinse the mouth with water and bicarbonate of sodium after each episode of vomiting, to neutralize the acids present in the oral cavity and to restore, as soon as possible, the physiological pH.

2.5. Level 4. Check-Up and post-natal procedures

In this level, further samples of plaque samples were collected for the last analysis. Professional oral hygiene sessions were performed and the patient's eating habits and nutrition status were verified.

Oral hygiene and feeding instructions for the period after childbirth, as well as oral hygiene procedures for the infant, were given.

The techniques of oral hygiene at home were resumed, also because the pregnant patient often tends to give less importance to their hygiene as focused on the child. Control sessions and motivational reinforcement were planned during which the following procedures were implemented:

- motivation of the patient to the recovery of oral hygiene;
- practical advice on quality and food intake and on the need for optimal nutritional intake. It was important to evaluate the composition and quality of diet as well as the methods of administration.

It was suggested to provide a fair amount of calories, proteins and vitamins to overcome the period of repair that the women's body must face; a control of the mother-child transmission of *Streptococcus Mutans* was also performed during the whole lactation period.

2.6. Quantitative analysis

The sample was diluted in 0.5 mm of sterile physiological solution 0.9%. Methylene blue dye 1% was used.

The preparation obtained was placed on the Thoma-Zeiss counter-chamber and analysed using an optical microscope (1200X magnification) to carry out the bacterial count. The Thoma-Zeiss chamber has a surface slide with a squared grid. Area of each box is known. The values of the Thoma-Zeiss chamber are $1/10 \text{ mm}^3$ of total volume and 1 mm^2 of total area.

It was possible to calculate the volume of bacterial suspension contained in each square, as the thickness between the lattice and the slide is known.

In this study, the 4 reticulates forming the main chamber diagonal were considered. By counting the cocci and the bacilli in this area and multiplying the total by four, it was possible to estimate the number of bacterial cells present in 1 mm^3 of the initial sample.

The legend of values is as follows:

- 0 = absence of bacteria
- 1 = rare bacteria (from 1 to 5)²³
- 2 = low concentration of bacteria (from 5 to 20)²³
- 3 = average bacteria (from 20 to 40)²³
- 4 = high concentration of bacteria (over 40)²³

2.7. Qualitative analysis

To perform the qualitative assessment, the initial portion of the sample, placed on the slide, was dried and fixed with heat.

Gram staining was then performed through the following steps: staining of bacterial forms with cristal-violet for 2–3 min, cover of the slide with a solution of iodine-iodide of potassium (Lugol) for two minutes for fix, positioning on the slide a solution based on alcohol and acetone for a minute, washing with water, cover slide with contrast based on fuch-sine diluted for a minute, lush with water and drying. The slide was analyzed with a 1200 X immersion microscope.

A numerical index has therefore been established for the qualitative analysis.

After collecting the samples, the following parameters were calculated: the trend of the plaque index, gingival bleeding index, the total number of cocci and bacilli, the amount of cocci and bacilli G+ and cocci and bacilli G- (Table 1).

3. Results

By performing a quantitative analysis, it was calculated that the average plaque index (Fig. 1) was $n = 48.1\%$ (T0), $n = 14.7\%$ (T1), $n = 18.4\%$ (T2) and $n = 18.9\%$ (T3). The plaque index score presents a downward trend, passing from 48.1% (T0) to 18.9% (T3). The number of total cocci (Fig. 2) was $n = 205.39$ (T0), $n = 57.5$ (T1), $n = 74.6$ (T2) and $n = 75.4$ (T3).

The number of total bacilli (Fig. 3) was $n = 62.7$ (T0), $n = 23.1$ (T1), $n = 25.3$ (T2), $n = 27.1$ (T3). The total values of cocci and bacilli were correlated and the average trend of the various samples was calculated (Fig. 4). By performing a qualitative analysis, the value of G+ cocci (Fig. 5) was $n = 2.7$ (T0), $n = 1.4$ (T1), $n = 1.4$ (T2) and $n = 1.5$ (T3). The value of G- cocci (Fig. 5) was $n = 2.3$ (T0), $n = 0.7$ (T1), $n = 1.1$ (T2) and $n = 1.1$ (T3). The value of G+ bacilli (Fig. 6) was $n = 1.6$ (T0), $n = 0.9$ (T1), $n = 1.2$ (T2) and

Table 1 Clinical parameters evaluated in T0, T1, T2, T3.

SAMPLE	P.I.	G.B.I.	N° cocci tot	N° bacilli tot	Cocci G+	Cocci G-	Bacilli G+	Bacilli G-
T0	47.4%	49	205.8	63.3	2.7	2.0	1.7	1.4
T1	14.3%	27	57.9	23.0	1.4	0.7	0.9	0.4
T2	18.8%	35	74.0	25.9	1.5	1.0	1.2	0.7
T3	19.4%	8	75.7	26.7	1.5	1.0	1.1	0.6

Legend of qualitative analysis G+ and G-:

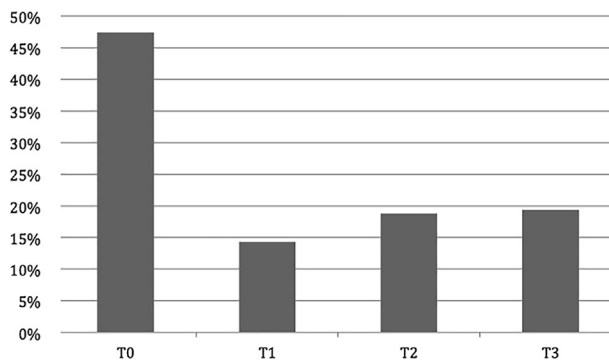
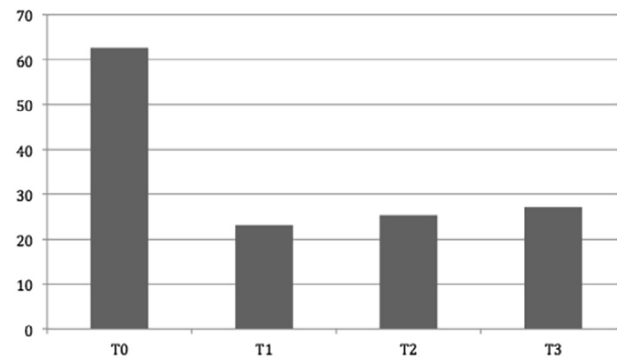
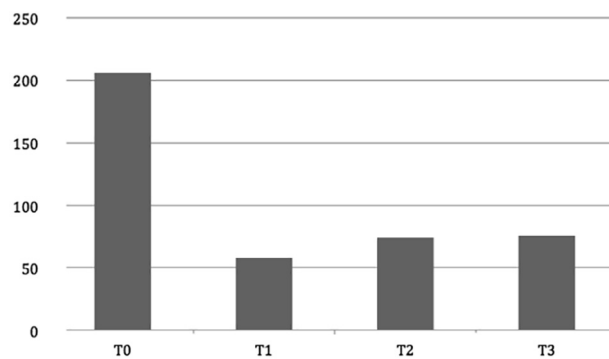
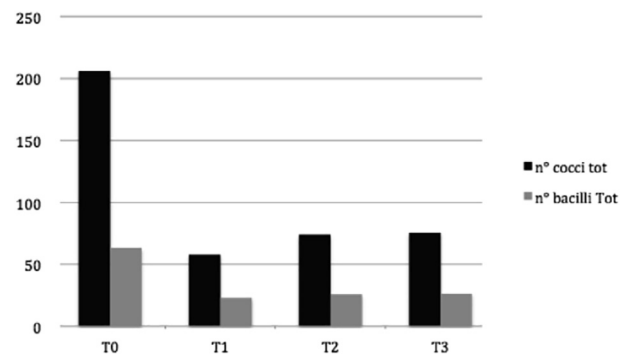
0 = absence of bacteria.

1 = rare bacteria (from 1 to 5)²³.

2 = few bacteria (from 5 to 20)²³.

3 = many bacteria (from 20 to 40)²³.

4 = very many bacteria (over 40)²³.

**Fig. 1** Average plaque index in T0, T1, T2, T3.**Fig. 3** Total number of bacilli in T0, T1, T2, T3.**Fig. 2** Total number of cocci in T0, T1, T2, T3.**Fig. 4** Correlation between total values of cocci and bacilli in T0, T1, T2, T3.

$n = 1.2$ (T3). The value of G- bacilli (Fig. 6) was $n = 1.3$ (T0), $n = 0.3$ (T1), $n = 0.7$ (T2) and $n = 0.7$ (T3).

The total values of cocci and bacilli were correlated and the average trend of the various samples was calculated. It can be seen that the total number of cocci and bacilli (Fig. 4) decreases during the protocol, so it can be concluded that the number of bacteria in oral cavity decreased considerably. In order to perform a qualitative analysis, a legend was used to simplify the calculation of bacteria. By analyzing qualitative data, it is noticeable that cocci G- and bacilli G- halved from T0 to T3. In addition it can be asserted that the number of cocci G+ and bacilli G+ shrunk considerably from T0 to

T3. Gingival bleeding index decreased from 49 to 8. The results of descriptive statistics are reported in Table 2.

4. Discussion

During pregnancy, many body modifications happen, such as salivary alterations, onset of caries, periodontal disease and epulis. Hormonal modifications alter the quality and quantity

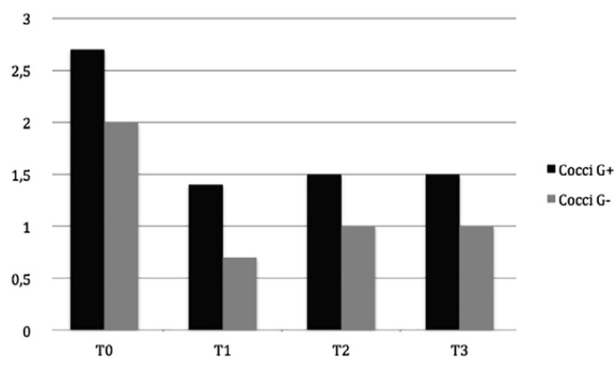


Fig. 5 Value of G+ and G- Cocci in T0, T1, T2, T3.

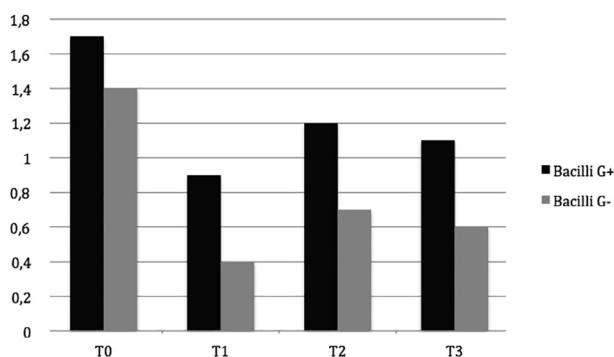


Fig. 6 Value of G+ and G- Bacilli in T0, T1, T2, T3.

of saliva. The saliva becomes more viscous, facilitating the adhesiveness of the bacteria to the tooth due to the increase in iron, calcium and potassium ions. Also the increase of mucin makes saliva more dense and adhesive.

The sialorrhea phenomenon seems more due to the feeling of nausea than to a real increase in salivary flow (Annan, 2005). Due to the action of estrogen-progestin hormones and the increase of acid-producing anaerobic bacteria following reduced oral hygiene, the salivary pH decreases (Masoni, 1991; Maspero et al., 2018 (a, b); Mignogna, 1992; Annan, 2005; Toselli, 1993; Silva de Araujo Figueiredo, 2017).

In pregnancy, however, the major cause of caries onset is the patient's difficulty in maintaining a regular schedule of appointments.

In fact, some studies have shown that the number of caries does not increase during and because of pregnancy, and that every relationship is indirect. The factors that lead to the formation of caries are the same during pregnancy as during other periods (Mignogna, 1992).

Only in women with nausea accompanied by prolonged vomiting over a long period, the pH can be lowered with demineralization and acid erosion of the tooth enamel (Goepel, 1991).

Between the 21st and the 40th week it is possible to find a reduction in calcium and phosphorus in the mother's saliva. Some authors report that this may temporarily cause the incidence of dental caries in the mother due to the absence of good oral hygiene (Salvolini, 1998; Kamate, 2017; Laine, 2002).

It is essential that a mother has no caries because it is directly from her mouth that the *Streptococcus mutans*, the bacteria responsible for the onset of caries, reaches the child's oral cavity (Lucchese, 2018; Carletto Körber, 2005).

The most frequent oral pathology in pregnancy is periodontal disease, which can occur, depending on the conditions of oral hygiene, with clinical pictures of varying severity from gingivitis to more severe lesions with periodontal pockets (Boggess, 2006). The less severe form of periodontal involvement is gingivitis, which is a reversible manifestation. The hormonal change causes an increase in vascularization, with consequent edema and therefore a tendency to bleed. Hormonal influence on the immune system contributes significantly to the etiology and pathogenesis of gingivitis (Malerba et al., 1987; Spadari, 2001).

Other studies have shown that, in the course of gestation, estrogen and progesterone levels are associated with gingivitis and increased quantities of *Prevotella intermedia*, a species of gram-negative anaerobic bacteria (Lindhe, 2006).

The risk of occurrence of periodontal disease is closely related to the secretion of placental and ovarian hormones; it starts to increase from the first quarter to the eighth month when the hormone production is higher (Masoni, 1991).

The etiopathogenetic factors contributing to the increased incidence of periodontal pathology in pregnancy are represented by changes in the composition of the subgingival bacterial flora, the alteration of the local immune response with increased gingival inflammation and the depression of the chemotactic and phagocytic response of neutrophils, of the production of prostaglandins induced by ovarian hormones. This pathology assumes a severity that is not directly associated with the patient's age, but with the conditions of oral hygiene that existed in the period preceding pregnancy (Masoni, 1991).

During pregnancy it is often possible to find the presence of gravidic epulis (Fig. 7), benign fibrous formations, normally asymptomatic (Annan, 2005).

They provide an ideal location for bacterial growth as they make it more difficult to remove plaque during oral hygiene maneuvers.

The etiology of the epulis can be attributed to the general effects of progesterone and estrogen (Ficarra, 2006). The

Table 2 Descriptive statistics of clinical parameters evaluated in the study.

Parameters	Values			
	Min	Max	Average	SD
Number of Cocci	57.9	205.8	103.35	68.76
Number of bacilli	23	63.3	34.72	19.11
P.I.	14.30%	47.40%	24.98%	0.15%
G.B.I.	8	49	29.75	17.44



Fig. 7 Gravidic Epulis.

therapy consists of the surgical excision of the lesion with the removal of the tissues up to the periosteum only if these formations are very annoying and are bleeding (Farronato et al., 1988a, 1988b), since in a number of cases they regress at the end of the pregnancy (Laine, 2002; Farronato et al., 1988a, 1988b; Boyarova, 2001; Orosz, 2007).

Research conducted by Farronato et al., shows the presence of dendritic istiocytes S-100 positive on 44 epulides studied through immunohistochemical procedures; the authors concluded that the presence of these cells, joined with immunitary response, may be important information in favour of inflammatory origin (Farronato et al., 1988a, 1988b).

Among the causes of low birth weight are premature birth and the delayed intrauterine growth.

More recently, among the causes of premature birth, along with genetic, haemographic, obstetric, toxic and nutritive risk factors, infectious factors have been called into question (Abati, 2007).

According to some authors, in the etiology of preterm birth, in addition to genital and urinary infections, oral infections also play an important role (Gibbs, 2001; Offenbacher 1998; Sánchez, 2007; López, 2005; Marin, 2005). Numerous studies on pregnant women have shown that the presence of periodontitis increases the risk of preterm birth (Martinez, 2012; Manau, 2008).

The timing of influence of periodontal disease on the course of pregnancy may improve the methodology of future investigations (Meqa, 2017). A systematic review of the literature states that the existence of a correlation between maternal parodontopathy and premature birth is probable, but not certain (López, 2007).

Maternal periodontal infection does not only affect the process of delivery, but also fetal growth, or altering placental function or having a direct effect on the foetus (Siqueira, 2007; Pretorius, 2007).

From the analysis of the literature, differences and discrepancies emerge on the role that oral hygiene has in relation to the outcome of childbirth. Most authors recommend treatment of periodontal disease in pregnant women, but do not guarantee that this would have a probable effect on reducing the risk of preterm birth (Marin, 2005; Iheozor-Ejiofor, 2017). However, the American Academy of Periodontology recommends that all pregnant women undergo a dental and periodontal

examination as a preventive measure (Tuchmann-Duplessis, 1984). Leverrier-Penna et al. studied the effects of ibuprofen on fetal ovaries ex vivo. Authors concluded that ibuprofen is currently only contra- indicated after 24 weeks of pregnancy (Leverrier-Penna, 2018).

Paracetamol is the anti-inflammatory drug of first choice for pregnant women because it does not interfere with bleeding time and is not teratogenic.

It is also useful to prescribe a chlorhexidine mouthwash that has the ability to eliminate potentially pathogenic bacterial strains for gingival-periodontal tissues.

For all these reasons, it is advisable to follow a preventive hygiene program like the one described in this paper. In fact the plaque index score decreased. By analyzing qualitative data, it is noticeable that cocci G- and bacilli G- halved from T0 to T3. In addition it can be asserted that the number of cocci G+ and bacilli G+ decreased considerably from T0 to T3. It can be seen that the total number of cocci and bacilli presents a downward trend, so it can be concluded that the number of bacteria in oral cavity decreased considerably (Maspero et al., 2018 (a, b); Farronato, 2014). These findings are supported by the results of previous studies that have demonstrated that women are in general unaware of oral health maneuvers during pregnancy and the effects that poor oral health may have on foetus (Sánchez, 2007; López, 2005; Marin, 2005; Martinez, 2012; Manau, 2008; Meqa, 2017; López, 2007; Siqueira, 2007).

5. Conclusions

The data collected in this study permits to conclude that the preventive hygiene protocol used in the Dental Hygiene Department of the University of Milan is a suitable method for the control of the bacterial plaque during pregnancy.

Periodontal infection is a potential risk factor for unfavorable pregnancy outcomes and preventive periodontal treatment is able to decrease risks like periodontal infections which, in extreme cases, can cause abortion or preterm birth.

The role of the dental hygienist assumes a crucial importance in instructing the pregnant woman on the correct home oral hygiene maneuvers and in motivating her during periodic checks to maintain a good periodontal health.

The pregnant woman should be carefully followed with periodic check-ups with the indications utilized in the levels (1-4) of operating procedures according to the protocol during the gestation period.

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Declaration of Competing Interest

Authors state to not have any conflict of interest.

Loan for work

Authors state to not have received institutional loans for this work.

Ethical approval

Ethical approval for this study n°421, 09/03/2016, Fondazione Ca' Granda, Ospedale Maggiore Policlinico, Milano, Italia.

The study was designed in accordance to principles of the Declaration of Helsinki for medical research involving human subjects.

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