Baština Akademije nauka i umjetnosti Bosne i Hercegovine

Cardiovascular diseases and oral health – the impact of pregnant women’s oral health on children’s cardiovascular health

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Chapter VI
RESEARCH PROJECT CARDIOVASCULAR DISEASES AND ORAL HEALTH – EFFECT OF ORAL HEALTH OF PREGNANT WOMEN ON CHILDREN'S CRDIOVASCULAR HEALTH

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Introduction

Adequate oral health reflects on and affects general health and quality of life. The oral cavity is an integral part of the human body and thus there is a great connection between oral health and systemic health. Not only do some systemic diseases such as diabetes, osteoporosis, HIV infection, trisomy 21 have a predisposition to periodontitis, but vice versa. Susceptibility to some systemic diseases is higher in patients with periodontitis than in healthy people: chronic periodontitis is a risk factor for future cardiovascular diseases, pregnant women with chronic periodontitis have more frequent preterm birth and lower birth weight. The explanation of the pathophysiological mechanisms of periodontal focus and systemic diseases is associated with increased levels of circulation of proinflammatory cytokines and prostaglandins originating from periodontal disease, gram-negative bacteria and their substances such as endotoxins emerging from subgingival biofilms. The dominant problems of everyday dental practice are: caries, periodontal diseases, occlusal anomalies, the relationship between oral and general health and a holistic approach to the patient. Caries and periodontal disease are of infectious etiology, and therefore the prevention of caries and periodontal diseases also means the prevention of odontogenic foci. Periodontal pocket is a risk factor for the occurrence or worsening of systemic – focal disease, because there is an always present infection; the flora of the pocket is diverse, massive, virulent and penetrates the soft membrane of the pocket, the pocket is under constant

mechanical stimulation during chewing, swallowing and speech, which all favors the penetration of bacteria into the circulation and the formation of transient bacteremia.

Interdisciplinary cooperation in eliminating the potential negative effects of periodontal infection will result in better systemic health.

Caries and periodontal disease – the most common and significant oral diseases, can cause and complicate many diseases – diseases of the cardiovascular system (infectious endocarditis, atherosclerosis, myocarditis and heart attack), respiratory system (pneumonia, chronic obstructive pulmonary disease, bronchial asthma, and lung abscess), neurological diseases (cerebral infarction and brain abscess), diabetes mellitus, rheumatoid arthritis, Alzheimer's disease, as well as other diseases (1, 2, 3, 4, 5).

Atherosclerosis is the basis for all cardiovascular diseases. There are studies that confirm that choral periodontitis increases the level of systemic inflammation when expressed by measuring C reactive protein (CRP) and other biomarkers. Periodontal pathogens can directly infect vascular endothelium and atherosclerotic plaque causing inflammation. Then, they can produce various virulent factors (adhesions, hemolysis) that have detrimental effects on the vascular system resulting in platelet adhesion and aggregation; in addition, lipid accumulations are formed and, along with cholesterol deposits, they contribute to the formation of atheromas. Treatment of chronic periodontitis reduces systemic inflammatory factors.

Pregnancy is a condition in which complex physical and physiological changes occur, all of which have significant effects on many organ systems. High levels of circulating estrogen during pregnancy have been associated with a high incidence of gingivitis and gingival hyperplasia (6). It is estimated that approximately 40% of pregnant women have some form of periodontal disease (5, 7).

Offenbacher et al. (1996) were the first to point out the possible connection between periodontal disease in pregnant women and the risk of giving birth to children of low gestational age or low birth weight (5). Numerous researchers point to the possible connection between periodontal disease in pregnant women and the risk of premature birth, and the birth of children with low birth weight (8, 9, 10, 11, 12). Studies show that pregnant women with periodontal disease have a 2-7 times higher risk of prematurity (8, 13). Morre et al. have found a large number of fetal deaths among mothers with periodontal disease (14).

Other authors have not found a link between the oral health of mothers and the risk of giving birth to premature babies or babies of low birth weight (15, 16).

However, focal infection can affect prematurity (birth before 37 weeks of gestation) and weight loss in newborns (weight <2,500 grams). Bacteremia of periodontal pathogens by transplacental route can lead to intrauterine infection. Proinflammatory cytokines release endotoxins lipopolysaccharides (LPS) that precipitate in preterm birth. Therefore, dentists should motivate, educate and instruct pregnant women to have a higher level of oral hygiene and to fix all orodental lesions in dental therapeutic procedures, especially periodontal pockets, for this reduces the number of premature births.

It is believed that gram-negative anaerobic bacteria present in periodontal tissues may be a source of endotoxin and lipopolysaccharides, leading to increased levels of inflammatory mediators – interleukin 6 (IL-6), interleukin 8 (IL-8), interleukin - 1 beta (IL-1β), and prostaglandin E (PGE) and tumor erosion factor – alpha (TNF-α), which are transmitted to the uterus, cervix and placenta causing prematurity or the birth of children of low birth weight (1, 7, 17). Jeffcoat et al., while investigating the association between periodontal disease in pregnant women and preterm birth in a group of 1,313 pregnant women, found that moderate to severe periodontal disease — diagnosed in early pregnancy — was associated with an increased risk of prematurity, independent of other traditional prematurity risk factors (18).

It has been found that mothers who gave birth to preterm infants or children with low birth weight had significantly elevated levels of PGE in gingival fluid compared to mothers who had term births or babies of desirable birth weight (17).

During pregnancy, there is an increased tendency to caries due to increased acidity of the oral cavity, increased consumption of refined sugars and insufficient oral hygiene (19). The tendency to caries during pregnancy is enhanced by periodic vomiting of pregnant women, which increases the acidity of the oral cavity, allowing the growth of cariogenic bacteria, demineralization of teeth and the progression of caries (19). Microorganisms and their toxins in pulp lesions caused by deep caries produce IL-1β, PGE and TNF-α, and as in periodontal disease, they cause premature birth or the birth of children of low birth weight (20, 21).

Cariogenic bacteria in children are most often transmitted by direct transmission through maternal saliva (5). Mothers who have a high titer of *Streptococcus mutans* in their saliva will largely transfer these bacteria to the

baby – vertical transfer, creating the preconditions for early childhood caries. Significant predictors of early childhood caries are certainly the time and frequency of bacterial transmission, children's tendency to susceptibility of bacterial accumulation on teeth, composition and flow of children's saliva, and the amount of refined sugar in baby food (1, 5, 22).

The incidence of premature births or low birth weight infants is between 5–18%, which depends on the geographical area or population characteristics (23). Considering the causes of premature births, or newborns of low birth weight are not known in more than 50% of cases, and in 50% of cases the most important causes are considered to be: socioeconomic factors, acute and chronic maternal diseases, multiple pregnancy, obstetric conditions and genetic diseases (23, 24).

Due to the immaturity of the organic systems, preterm infants and newborns of low birth weight fall under the group of endangered infants – where complications due to prematurity are the leading cause of death in children under five year of age (23, 24). It is very important to discover the risk factors that can determine the risk of prematurity or the birth of newborns of low birth weight, where by knowing the risk factors, it is possible to significantly eliminate or reduce the risk of giving birth to premature infants or children of low birth weight and reduce the rate of perinatal mortality or possible complications.

Premature babies or low birth weight newborns show a higher incidence of cardiovascular risk factors (obesity, hypertension and dyslipidemia) and type 2 diabetes mellitus (25). Animal and epidemiological studies show that intrauterine elevated glucocorticoid levels cause lifelong programming of the hypothalamic-pituitary-adrenal axis, which plays a key role in increasing the incidence of cardiovascular risk in premature and low birth weight infants (26).

There is a hypothesis on the possible connection between chronic inflammation (*Chlamydia pneumoniae*, *Helicobacter pylori*, *Cytomegalovirus*, *Ebstein-Barr* virus, etc.) and atherosclerotic cardiovascular disease, but the precise pathogenesis is not completely clear (27, 28).

In addition to the role of the oral microbiome (microbiome: all microorganisms, their genome and interaction in a given environment) as a risk factor for prematurity or the birth of low-birth-weight infants, oral microorganisms may also be the cause of chronic inflammation that may be a risk factor for atherosclerotic (29, 30).

Adequate prenatal care should also include the protection of the oral health of pregnant women, and the pregnant women should be reminded of

the importance of practicing regular oral hygiene and the need for periodic or, if necessary, more frequent dental examinations. Possible dental interventions, such as radiography of the teeth, are best suited after the end of organogenesis, or in 2nd or 3rd trimester of pregnancy (5, 7, 31).

We believe that in the daily practice there is not enough screening of the oral health status of pregnant women, where screening the oral health status of as many pregnant women as possible, including assessment of oral hygiene, could identify pregnant women with caries or periodontal disease.

Timely dental treatment could reduce the incidence of caries and periodontal disease in pregnant women, and possibly reduce the incidence of prematurity and low birth weight infants, early childhood caries and predictors of premature atherosclerotic cardiovascular risk (increase in body mass index, blood pressure and carotid intima-media complex thickening).

2. Research hypotheses and objectives

2.1. Research hypotheses

In this study, we set the following hypotheses:

H_1 – A considerable number of pregnant women have inadequate oral health.

H_2 – Insufficient number of visits to the dentist, inadequate oral hygiene and consumption of unhealthy foods lead to the occurrence of caries and periodontal disease in pregnant women.

H_3 – In a certain number of pregnant women with poor oral health status, pregnancy results in births of premature and/or babies of low birth weight.

H_4 – In the cohort of children who were born prematurely or had low birth weight, a certain number of children aged three (4) have a higher body mass index, and a higher value of systolic and diastolic blood pressure, as well as carotid intima-media complex thickening with incipient signs of cardiovascular disease.

H_5 – There is an inappropriate knowledge, opinion and practice of pregnant women and practitioners (dentists and gynecologists) about the state of oral health and its impact on pregnancy and the overall health of newborn babies.

Knowledge, opinion and practice of both pregnant women and colleagues (dentists and gynecologists) about the state of oral health and its impact on pregnancy and overall health of newborn child, is quite poor in the Balkans and the genesis of oral health problems in our region.

2.2. Research objectives

Insufficient insight into possible pathological implications of the oral health status of pregnant women on the premature manifestation of cardiovascular risk factors in children has started research, by which we want to:

1) evaluate the general health condition and life habits of pregnant women:
– measure the body mass index of pregnant women – data in medical files
– determine whether pregnant women have a certain chronic disease (cardiovascular disease, diabetes mellitus, kidney and other chronic diseases) or whether they are taking any medication
– analyze eating habits, physical activity, as well as the presence of smoking and alcohol consumption in pregnant women
– determine the course of pregnancy, and whether there were any complications during the pregnancy.

2) determine the basic characteristics of oral health protection during pregnancy:
– frequency of visits of pregnant women to the dentist
– adequacy of oral hygiene
– oral health status of pregnant women.

3) analyze the basic characteristics of the newborn:
– evaluate gestational age and birth weight of newborns
– analyze the method of delivery (natural or caesarean section).

4) determine the basic characteristics of the general pediatric condition and the oral health status of children:
– analyze children's eating habits (diet in infancy, whether dietary guidelines are followed in children)
– determine the body weight and length/height of children
– calculate the body mass index of children
– determine the value of blood pressure in children
– determine carotid artery intima-media complex thickening using the ultrasound method
– determine when the child's first visit to the dentist was
– assess the oral health status of children
– determine the impact of the oral health status of mothers on the oral health status of children.

5) determine the knowledge, opinion and practice of pregnant women, as well as practitioners (dentists and gynecologists) about oral health, the impact of oral health on pregnancy and the health of a newborn baby
6) determine the predictors of maternal oral health status
7) determine the correlation between the oral health status of mothers with gestational age and the birth weight of newborns
8) determine the correlation of maternal oral health status with predictors of premature cardiovascular risk in children (overweight, elevated systolic and diastolic blood pressure, as well as increased carotid artery intima-media complex thickening determined by carotid artery ultrasound).

3. Respondents and methods

3.1. Area of research

The survey would be conducted in the Federation of Bosnia and Herzegovina – Canton Sarajevo and the Republic of Croatia (Zagreb and Split), considering the proportional distribution of respondents.

3.2. Respondents

The minimum number of respondents required for adequate statistical analysis of the research variable is 385, with an error of 5% and 95% confidence interval, determined by a statistical calculator (32).

The research is designed as a cohort study – it will include mothers/pregnant women, who will be selected by random selection (randomized sampling).

The research will be conceived as a multidisciplinary approach – cooperation of gynecologists and obstetricians, dentists, pediatricians, radiologists and cardiologists, for three or four years.

During the usual gynecological and obstetric examination (1st trimester, and if necessary 2nd and 3rd trimester of pregnancy) pregnant women would be suggested to consult a dentist in order to assess the status of oral health.

Based on the insight into the health documentation of pregnant women, the general health condition will be assessed – pregnant women with cardiovascular diseases diabetes mellitus, kidney diseases or any other chronic diseases will not be included in the research. Survey testing will assess the life habits of pregnant women (eating habits, physical activity,

alcohol consumption, drugs and smoking).

After birth, children would be classified into term newborns and premature infants, infants of desirable body weight and infants of low body weight and would be monitored until the third or fourth year of life.

During systematic examinations (first month of birth, first year and third and fourth year), pediatricians would then evaluate the basic characteristics related to pregnancy and childbirth, analyze children's eating habits, anthropometric parameters, check the blood pressure, while radiologists would check the value of the carotid artery intima-media complex thickening, and cardiologists would evaluate using echocardiogram the hemodynamic status of children.

The research would include children in good health, which means that children with congenital anomalies or certain chronic diseases would be excluded from the study.

Dentists would assess the dental health status of pregnant women and children and evaluate the adequacy of oral hygiene of pregnant women and children.

The research will be based on the principles of the 1975 Declaration of Helsinki and its amendments from 2008.

In order to implement the ethical and bioethical principles of research, the consents of the relevant Ethics Committees/Commissions will be sought.

Voluntary participation by pregnant women and their children will be confirmed by signing an informed consent.

3.3. Research methods

After signing the informed consent, and getting acquainted with mothers/pregnant women using the research methodology, we would perform the following on mothers/pregnant:

– surveying,
– dental examination.

For children we would perform the following:

– surveying
– anthropometric measurement
– checking blood pressure values
– measuring carotid artery intima-media complex thickening using two-dimensional color doppler ultrasound

– two-dimensional Color Doppler echocardiographic evaluation of the cardiovascular system
– dental examination.

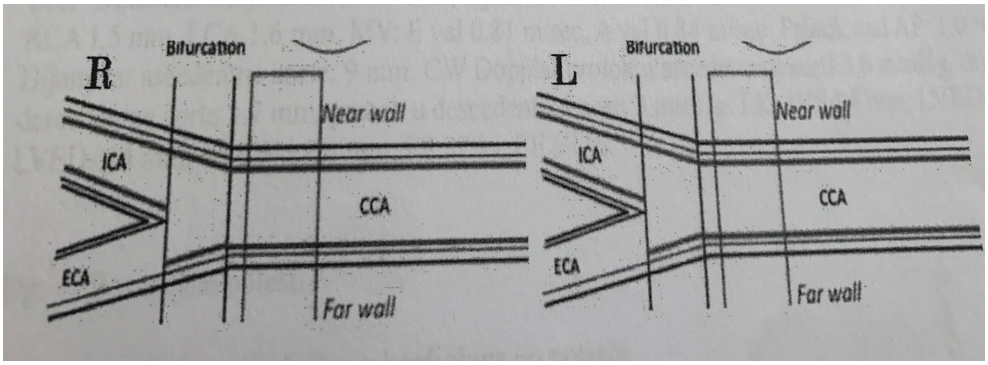


Figure no.1. Schematic diagram of measuring the carotid artery intima-media complex thickening by Color Doppler ultrasonography; CCA common coronary artery, LCA left carotid artery, ECA external carotid artery; R – right; L – left

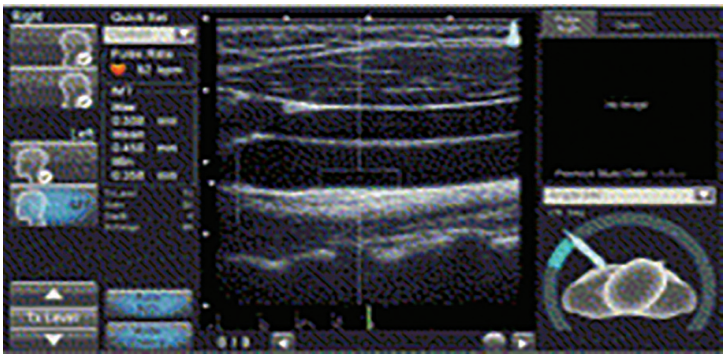


Figure no. 2. Ultrasonography of carotid arteries A

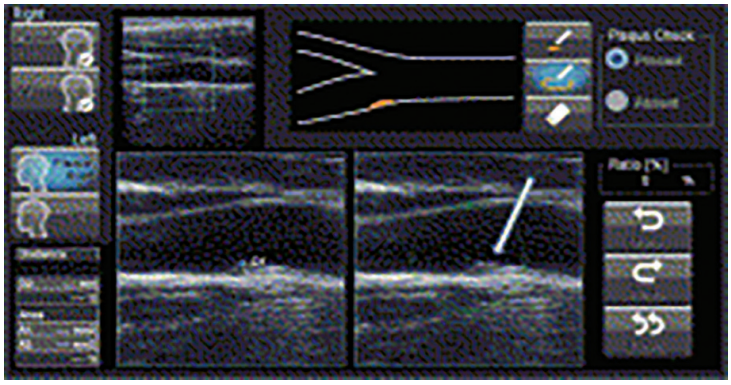


Figure no. 3. Ultrasonography of carotid arteries B.

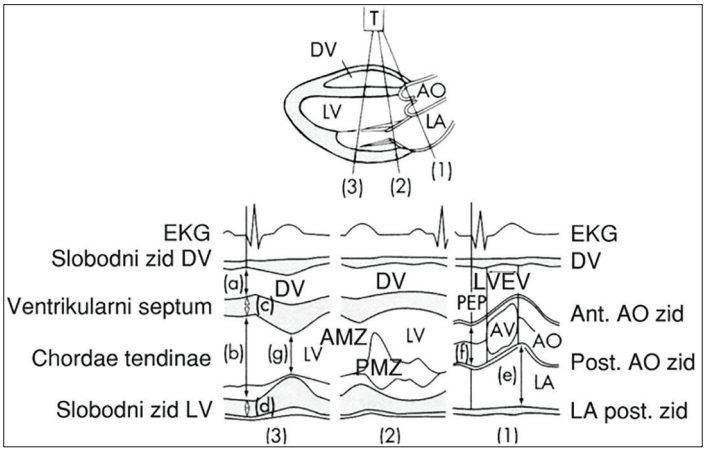


Figure no. 4. Cross section of the left side of the heart through the parasternally long and short axis; a – dimensions of DV; b – end-diastolic dimensions LV; c – thickness of ventricular septum, d –thickness of posterior wall; e – dimension LA; f – aortic dimension; g – systolic dimensions LV, AMZ – anterior mitral valve; LV EV – LV ejection time, PEP – pre-ejection period.

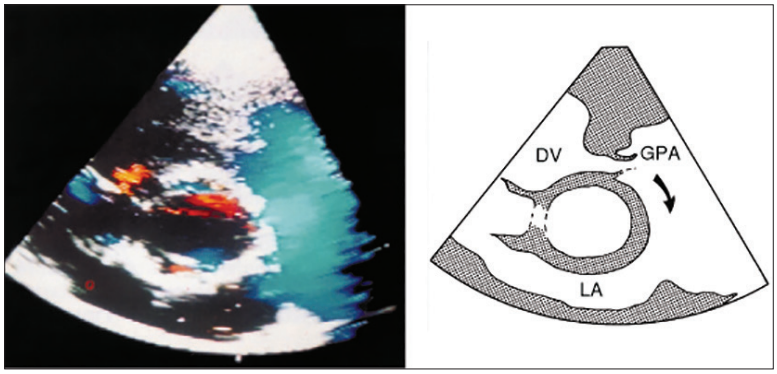


Figure no. 5. Color Doppler: short axis of systole transverse: flow through the main pulmonary artery (GPA); DV – right ventricle; LA – left atrium

3.3.1. Surveying of mothers/pregnant women

Each respondent will have her own encrypted ID number.

A survey questionnaire that would be adapted to this research would contain the following:

– general data (mother's age, city, educational status, employment status and socio-economic status of the family);

- data from personal anamnesis (mother's health condition: whether she suffers from a chronic disease, whether the mother is taking certain medications, data on pregnancy, number of previous births, the manner of giving birth, TM, TV, BMI)
- data on eating habits (number of daily meals, consumption of fruits, vegetables and other foods, consumption of sweets and other foods with refined sugars and consumption of soft drinks);
- data on harmful habits (smoking, narcotics and alcohol);
- data on physical activity;
- data on oral health (when was the last visit to the dentist before pregnancy, in what trimester of pregnancy she visited the dentist, knowledge, opinion and practice of oral hygiene, whether the mother was advised to maintain dental health ...).

QUESTIONNAIRE

"THE IMPACT OF PREGNANT WOMEN'S ORAL HEALTH ON CHILDREN'S CARDIOVASCULAR HEALTH"

Questionnaire for the mother

IDB	|__|__||__|				
Survey date:	|__|__| / |__|__| / 2017.				
Health institution:					
City/Town:					
Country:					
Contact telephone:	E-mail:				
1. GENERAL INFORMATION					
1.1. Your year of birth:					
1.2. Your educations status:	Primary school ☐	High School ☐	Junior College ☐	University ☐	☐ Other:
1.3. Your work status:	Employed ☐		Unemployed ☐		
1.4. According to your estimation, your family economic status is:	Below average ☐		Average ☐		Above average ☐
1.5. Besides this child, you:	Do not have other children ☐		I have younger child(ren) (state number) ☐ ___		I have older child(ren) (state number) ☐ ___
2. INFORMATION ON PREGNANCY					
2.1. How did the pregnancy go?	Good ☐		Complications ☐ (which:)		
2.2. Diagnosed illnesses during pregnancy?			None ☐	Rare/mild ☐	Often/severe ☐
2.3. Do you consume alcohol during pregnancy?	Yes ☐		Sometimes ☐		No ☐
2.4. Do you smoke during pregnancy?	Yes ☐		Sometimes ☐		No ☐
Do you take medications during pregnancy?	Yes ☐		No ☐		
**If you answered "Yes", please state which medications:*					
3. EVALUATION OF ORAL HEALTH KNOWLEDGE (Please provide only one answer for each question!)					
3.1 In your opinion, how often do you need to brush your teeth?	At least 1x day ☐	At least 2x day ☐	After each meal ☐	Not necessary to brush teeth every day ☐	I don't know ☐
3.2. How long should you brush your teeth?	Up to 1 min ☐	1-3 minutes ☐	More than 3 minutes ☐	I don't know ☐	
3.3. Is it necessary to use dental floss to thoroughly clean your teeth?	Yes ☐	No ☐	I don't know ☐		
3.4. Does toothpaste need to contain fluoride?	Yes ☐	No ☐	I don't know ☐		
4. EVALUATION OF THE OPINION ON ORAL HEALTH (Please provide only one answer for each question)					
4.1. Can the condition of the teeth and oral cavity have a negative impact on your general health?	I agree ☐	I don't agree ☐	I am not sure ☐		
4.2. Regular control of teeth and oral cavity is important to prevent caries and periodontal diseases?	I agree ☐	I don't agree ☐	I am not sure ☐		
4.3. Poor state of your dental health can lead to premature birth or the birth of a child of low birth weight?	I agree ☐	I don't agree ☐	I am not sure ☐		

4.4. Poor state of your dental health can lead to your' child having dental problems or some other diseases?	I agree ☐	I don't agree ☐	I am not sure ☐
4.5. Are regular dental checkups necessary during pregnancy?	I agree ☐	I don't agree ☐	I am not sure ☐
4.6. Dental interventions are safe during pregnancy?	I agree ☐	I don't agree ☐	I am not sure ☐

5. EVALUATION OF ORAL HEALTH PRACTICE
(Please provide only one answer for each question!)

5.1. How often do you brush your teeth?	In the morning only ☐	In the evening only ☐	In the morning and in the evening ☐	After every meal ☐	I do not brush my teeth every day ☐
5.2. How long do you brush your teeth?	1 minute ☐		1-3 minutes ☐	>3 minutes ☐	
5.3. Do you also use dental floss in addition to tooth brush and toothpaste?	Yes ☐	No ☐			
5.4. Do you use antibacterial mouth wash?	Yes ☐	No ☐			
5.5. Do you use chewing gum?	Yes ☐	No ☐			
5.6. If you use chewing gum, state which chewing gum you use?	Chewing gum with xylitol ☐	Chewing gum with sugar ☐	Chewing gum without sugar ☐	I use any chewing gum ☐	
5.7. How many times did you have a regular visit to the dentist over the past year?	1x ☐	2x ☐	I did not visit a dentist ☐	☐ I cannot remember	
5.8. Most usual reason to visit a dentist is?	Regular dental check-up ☐	Tooth pain ☐	Tooth extraction ☐	Fixing a tooth ☐	Other reasons ☐ State:
5.9. How many daily meals do you have?	1-2 meals ☐	3 meals ☐	4 meals ☐	5 meals ☐	>5 meals ☐

5.10. How often do you consume the following foods?

Food	≥ 2 per day	1x per day	Few times a week	1x week	Rarely or never
Milk or dairy products					
Fruit					
Vegetables					
Fruit juice					
Non-alcohol beverages					
Sweets (sugar, candy)					
Sweets (cakes, cookies, chocolate)					
Honey, marmalade, various spreads					
Toffee					
Chips, sticks and other snack					
Meat					
Fish					
Eggs					

5.11. My source of information on oral health is most often:	My dentist ☐	Means of public information (newspapers, radio, TV) ☐	Internet (web pages) ☐	Friends, acquaintances ☐

Signature:

_____

Thank you for participation!

ID NUMBER OF THE PREGNANT WOMAN:	
NUMBER OF PATIENT'S HEALTH RECORDS:	
EXAMINATION DATE:	

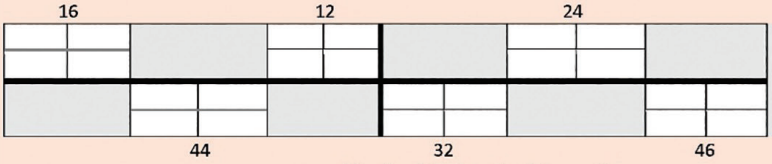
A. GENERAL RISK PARAMETERS:

1. FORM OF FLUORIDE USED:	In drinking water ☐	Tablet from 4th month of pregnancy ☐	Tablet occasionally ☐	Did not use ☐
2. MOTHER'S ORAL HEALTH:	0 - Neg. ☐	1 – Treated mouth ☐	2 - Untreated mouth ☐	
3. FATHER'S ORAL HEALTH:	0 - Neg. ☐	1 – Treated mouth ☐	2 - Untreated mouth ☐	

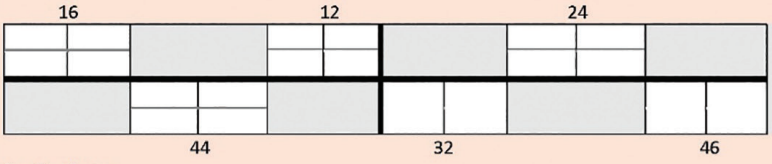
B. SPECIFIC RISK PARAMETERS:

B1 ***Oral hygiene assessment***
Plaque index (Silness & Loe)

Prior to health education intervention



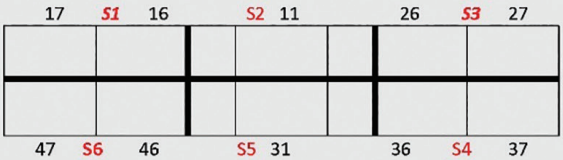
After health education intervention



0 – No plaque
1 - Along the edge of the gingiva (detection – using a probe or staining)
2 - In gingival sulcus / pocket or visible on tooth and gingival margin
3 - Large

B2 – ***Periodontal assessment and necessary treatments***
CPITN (*Community Periodontal Index of Treatment Needs*)

Prior to health and education intervention

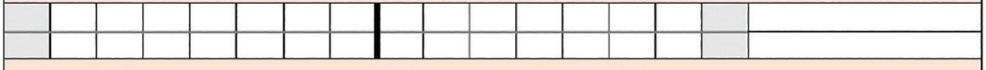


0- Healthy parodontium
1- Bleeding (probing <3,5mm)
2- Limestone (probing <3,5mm)
3- Periodontal pocket (4-5 mm)
4 – Periodontal pocket (>6mm)

0- No need for treatment
1- Instruction by OH
2- 1+Scraping, polishing of the root
3- 1+ Scraping, polishing of the root
4- 1 Scraping, polishing of the root, periodontal surgery

After health and education intervention:

B3 - ***Status of the tooth***
KEP index



K – Caries
E – Tooth extracted
P – Seal

KEP =

QUESTIONNAIRE

"THE IMPACT OF PREGNANT WOMEN'S ORAL HEALTH ON CHILDREN'S CARDIOVASCULAR HEALTH "

Questionnaire for the child

IDB	|__|__||__|				
Survey date:	|__|__| / |__|__| / 2017				
Health institution:					
City/Town:					
Country:					
Contact telephone:	E-mail:				
1. GENERAL INFORMATION					
1.1. Your child's date of birth:					
1.2. Child's sex:	Boy ☐	Girl ☐			
2. INFORMATION ON MOTHER'S PREGNANCY AND LABOR (Please provide only one answer for each question!)					
2.1. Pregnancy in weeks?					
2.2. Child was born:	On time ☐	Preterm ☐	Premature ☐		
2.3. Were there complications during pregnancy?	N ☐	Y ☐	Which:		
2.4. How did birth end?	Natural ☐	C-section ☐	Forceps ☐	Vacuum extraction ☐	
2.5. Were there complications during labor?	N ☐	Y ☐	Which:		
2.6. **APGAR score**	1 minute		5 minutes		
2.7. **Birth weight (BW) of the child**		**Birth length (BL) of the child**			
3. INFORMATION ON INFANT PERIOD (Please provide only one answer for each question!)					
3.1 Did the child have any of the following during the first year of life?					
- Congenital heart disease?	No ☐	Yes ☐			
- Other chronic diseases?	No ☐	Yes ☐			
*If your answer to the question above was "Yes", please state which diseases:					
3.2. Was your child on a natural diet / breastfeeding during the first year of life?	Did not breastfeed or breastfed only a couple of months ☐				
	Exclusive breastfeeding 6 months with continued breastfeeding and introduction of non-dairy foods after 6 months of age (fruits, vegetables, meat...) ☐				
	Combination of breastfeeding and milk formula and introduction of non-dairy foods after 4 months of age ☐				
	Milk formula and introduction of non-dairy foods after 4 months of age ☐				
EVALUATION OF ORAL HEALTH KNOWLEDGE (Please provide only one answer for each question)					
4.1. If the mother has caries or poor oral health – can this have an impact on the occurrence of caries in children?	Yes ☐	No ☐	I don't know ☐		
4.2. Is it necessary to treat caries in young children?	Yes ☐	No ☐	I don't know ☐		
4.3. When should a child make a first visit to the dentist?	Between 6 and 12 months of child's life ☐				
	When child is 1 ☐				
	When child is 2 ☐				
	When child is 3 ☐				
	When child should start first grade of school ☐				
	I don't know/I am not sure ☐				

5. EVALUATION OF THE OPINION ON ORAL HEALTH (Please provide only one answer for each question!)			
5.1. Can children with caries in early childhood later have a high chance of having caries of permanent teeth?	Yes ☐	No ☐	Not sure ☐
5.2. Can frequent and prolonged night meals with milk formula lead to childhood caries?	Yes ☐	No ☐	Not sure ☐
5.3. Can frequent and prolonged feeding of sweetened beverages (tea, juice) leas to caries in children?	Yes ☐	No ☐	Not sure ☐
5.4. Can mother's kisses and her tasting baby's food with the same spoon cause caries in children?	Yes ☐	No ☐	Not sure ☐

6. EVALUATION OF ORAL HEALTH PRACTICE (Please provide only one answer for each question!)	
6.1. Before brushing baby's teeth, did you clean your baby's gums or mouth with a piece of gauze soaked in water or special moist paper tissue – after baby's last meal for the day?	Daily ☐ Seldom ☐ Never ☐
6.2. When did you start brushing your child's teeth with toothpaste containing fluoride?	Immediately after teething ☐ (baby's month:) At the age of (state the month) ☐ Deciduous teeth do not have to be brushed ☐ I don't know ☐
6.3. How many times per day do you brush your child's teeth with fluoride toothpaste?	In the morning only ☐ In the evening only ☐ In the morning and in the evening ☐ Sometimes during a week ☐ I do not brush child's teeth ☐

6.4. At what age (months) did child have its first visit to a dentist?		None ☐
6.5. During the past year, how many times did the child visit a dentist?	1x ☐ 2x ☐ None ☐	Numerous (number) ☐
6.6. Most frequent reasons to visit a dentist?	Regular dental check-up ☐ Tooth pain ☐ Tooth extraction ☐ Fixing a tooth ☐	Other reasons ☐ State:
6.7. How many daily meals do you have?	1-2 meals ☐ 3 meals ☐ 4 meals ☐ 5 meals ☐	>5 meals ☐
6.8. What drink does the child most often consume?	Milk ☐ Water ☐ Tea ☐	Juice ☐

6.9. How often does the child consume the following foods?

Food	≥ 2 per day	1x per day	Few times a week	1x week	Rarely or never
Milk or dairy products					
Fruit					
Vegetables					
Fruit juice					
Non-alcohol beverages					
Sweets (sugar, candy)					
Sweets (cakes, cookies, chocolate)					
Honey, marmalade, various spreads					
Toffee					
Chips, sticks and other snack					
Meat					
Fish					
Eggs					

6.10. My source of information on oral health is most often:	My dentist ☐	Means of public information (newspapers, radio,	Internet (web pages) ☐	Friends, acquaintances ☐

3.3.2. Dental examination: diagnosing initial caries, periodontal examination of the gingival pocket depth and an overall examination of the oral cavity (detailed clinical parameters for the evaluation of dental status will be defined according to WHO recommendations).

3.3.3. *Surveying the children*

Each respondent (child) will have its own encrypted ID number.
A survey questionnaire that would be adapted to this research would contain the following:

- general data (child's age and sex, city);
- data from personal anamnesis (gestational age, birth weight (BW), birth length (BL), child's health)
- data on eating habits (natural or artificial nutrition in infancy, practicing dietary guidelines, excessive consumption of fruit juice, cakes and sweets);
- data on physical activity;
- data on oral health (Prior the planned pregnancy, did you purposely fix all pathological processes in the oral cavity? When you made an appointment with the dentist, did you point out that you were pregnant? X-rays of low-dose dental X-rays are absolutely contraindicated in all stages of pregnancy; During pregnancy you should not take out a tooth and have local anesthesia?; During pregnancy you increased the level of oral hygiene?; How many preventive dental examinations are recommended during pregnancy?; My gynecologist did not recommend dentist intervention with local anesthesia?; At what age was the first visit to the dentist?; Do you practice oral hygiene?; Was the mother advised to maintain the child's dental health before planning a pregnancy?).

3.3.4. *Anthropometric measurement*

Anthropometric measurement will include measuring body weight and body height while following the usual standard guidelines of anthropometric measurement.

We will calculate the body mass index of respondents using WHO Anthro software (33), and the obtained values will be compared according to the guidelines of the WHO - Body Mass Index – for gender and age (34, 35).

3.3.5. *Measuring blood pressure in children*

At the age of three (four), we will measure the value of children's blood pressure according to the standardized procedure using the auscultation method (Riva-Rocci-Korotkoff) with an appropriate cuff.

3.3.6. Measuring intima-media complex using ultrasound method, two-dimensional Color Doppler ultrasonography

3.3.7. Two-dimensional Color Doppler echocardiographic evaluation of the cardiovascular system.

3.3.8. Dental examination to diagnose initial caries, periodontal examination to diagnose the depth of the gingival pocket and a comprehensive examination of the oral cavity.

3.4. Evaluation of variables

In addition to the evaluation of the educational status of mothers, additional evaluation will be done in terms of socio-economic status of families, working status of mothers, and certain clinical variables of mothers/pregnant women and children.

3.4.1. *Classification of children's gestational age and birth weight*

Children with gestational age <37 weeks will be classified as preterm babies, and those with birthweight <2,500 g will be classified as low birth weight children.

3.4.2. *Classification of oral health (for mothers and children – milk dentication)*

Based on common dentistry classifications, fellow dentists will supplement the exams with dental status, periodontal status, gingival bleeding, etc.

3.4.3. *Classification of body mass index of children*

Using WHO criteria (34, 35).

3.4.4. *Classification of blood pressure in children*

After measuring blood pressure (3x), we will calculate the mean of systolic and diastolic blood pressure, and it will be compared with standardized

criteria for evaluating blood pressure in children and adolescents – according to the Fourth Report on Diagnosis, Treatment and Evaluation of High Blood Pressure in Children and Adolescents (36, 37).

3.4.5. *Classification of carotid artery intima-media complex*

Based on the usual classification of carotid blood vessel intima-media complexes using the ultrasound method.

3.4.6. *Classification of possible early changes/diseases of the cardiovascular apparatus using the ultrasonic two-dimensional Color Doppler echo sonographic method*

3.5. Statistical data processing

The data obtained from the survey will be entered into a database (MS Access or MS Office) and will be evaluated by an appropriate statistical program in consultation with a statistician, who will use appropriate parametric and non-parametric tests in statistical data processing.

In the first phase of statistical processing, which is the presentation of sample characteristics, standard methods of descriptive statistics will be used (graphs, measures of central tendency, measures of dispersion).

We will then examine the strength of the correlation between maternal oral health status and all other variables (gestational age, family economic status, level of education, possibly whether mother lives in urban/suburban or rural area, body mass index, eating habits, alcohol consumption, smoking, regularity of visits to a dentist, and regular oral hygiene practices).

We will also determine the strength of the correlation between the oral health status of mothers and the risk of premature birth and the incidence of children of low birth weight. In this context, we will evaluate whether some other variables are predictors of the incidence of premature babies or children of low birth weight.

The statistical significance of the differences between the values of anthropometric variables, the values of systolic and diastolic blood pressure, and possibly the thickness of the carotid artery intima-media complex, the variables in term infants with the desired birth mass vs. children born prematurely and children of lower birth weight. We will determine whether the status of oral health of mothers and the premature birth of children and

birth of children of low birth weight is a predictor of elevated values of body mass index, blood pressure and thickening of the carotid artery intima-media complex.

4. Expected results

The results of the research will show that a certain number of mothers have a poor (inappropriate) oral health status due to insufficient practice of oral health hygiene, poor practice of appropriate dietary guidelines and insufficient visits to the dentist.

The research will determine that a certain number of mothers with poor oral health status give birth to premature babies and children of low birth weight.

We expect that children of mothers with poor oral health status at the age of 3 (4) also have poorer oral health status compared to children whose mothers with good oral health status.

The group of children, born prematurely or born with low birth weight, at the age of 3 (4) years have a higher body mass index for their age and sex, higher systolic and diastolic blood pressure and greater thickness of carotid intima-media complex compared to the desired values of these parameters in term babies and babies with desirable birth weight with possible incidental indications of cardiovascular disease including atherosclerotic, compared to the control group.

Anonymous survey for the dentist and the gynecologist:

- As a dentist, I avoid applying local anesthesia with adrenaline to pregnant women in all months of pregnancy?
- I avoid any type of X-ray in pregnant women ?
- Pulling out a tooth under pain in pregnant women is not recommended during pregnancy?
- I avoid treating gingivitis in pregnant women at all stages of pregnancy?
- Trepanation of the tooth causing acute odontogenic infection and incision of the abscess are not recommended when woman is pregnant?

Study timeline:

Pregnant woman – gynecologist – dentist – pediatrician – radiologist – cardiologist – epidemiologist

References

1. Pezelj-Ribarić i sur. Oralno zdravlje – uvjet za opće zdravlje. Rijeka; Medicinski fakultet Sveučilišta u Rijeci; 2013.
2. Teles R, Wang CY. Mechanisms involved in the association between periodontal diseases and cardiovascular disease. Oral Dis 2011;17:450–461.
3. Zoellner H. Dental infection and vascular disease. Semin Thromb Hemost 2011;37:181–192.
4. Kamer AR, Craig RG, Dasanayake AP, Brys M, Glodzik-Sobanska L, de Leon MJ. Inflammation and Alzheimer's disease: possible role of periodontal diseases. Alzheimers Dement 2008;4:242–250.
5. Boggess KA, Edelstein BL. Oral health in women during preconception and pregnancy: implications for birth outcomes and infant oral health. Matern Child Health J 2006; 10 (5 Suppl):S169-174.
6. Hemaltha V, Manigandan T, Sarumathi T, Aaarthi Nisha V, Amudhan A. Dental considerations in pregnancy − a critical review on the oral care. J Clin Diagn Res 2013; 7:938.
7. American College of Obstetricians and Gynecologists. Oral health care during pregnancy and through the lifespan. Committee Opinion No. 569. Obstet Gynecol 2013;122:417–422.
8. Offenbacher S, Katz V, Fertik G, Collins J, Boyd D, Myynor G i sur. Periodontal infection as a possible risk factor of preterm low birth weight. J Periodontal 1996;67:1103-1113.
9. Soroey M, Avanbadejo P, Savage K, Oluwole A. Association between periodontal disease and pregnancy outcomes. Odontostomatol. Trop. 2015; 38 (152):5-16.
10. Turton M, Africa CW. Further evidence for periodontal disease as a risk indicator for adverse pregnancy outcomes. Int Dent J. 2016 Dec 17. doi: 10.1111/idj.12274.
11. Trivedi S, Lal N, Singhal R. Periodontal disaease during pregnancy. J Orofacial Sci 2015; 7:67.
12. Govindaraju P, Venugopal S, Shivakumar MA, Sethuraman S, Ramaiah SK, Mukundan S. Maternal periodontal disease and preterm birth: A case-control study. J Indian Soc Periodontol. 2015;19(5):512-515. doi: 10.4103/0972-124X.164751
13. Reza Karimi M, Hamissi JH, Naeini SR, Karimi M. The Relationship Between Maternal Periodontal Status of and Preterm and Low Birth Weight Infants in Iran: A Case Control Study. Glob J Health Sci. 2015 ;8(5):184-188. doi: 10.5539/gjhs.v8n5p184.
14. Moore S, Ide M, Coward PY, Randhawa M, Borkowska E, Baylis R, Wilson RF. A prospective study to investigate the relationship between periodontal disease and adverse pregnancy outcome. Br Dent J. 2004 Sep 11;197(5):251-258.
15. Ali TB, Abidin KZ. Relationship of periodontal disease to pre-term low birth weight infants in a selected population--a prospective study. Community Dent Health. 2012; 29(1):100-105.
16. Davenport ES, Williams CE, Sterne JA, Murad S, Sivapathasundram V, Curtis MA. Maternal periodontal disease and preterm low birthweight: case-control study. J Dent Res. 2002;81(5):313-318.
17. Offenbacher S, JaredHL, O′Reilly PG, Wells SR, Salvi GE, Lawrence HP et al. Potential pathogenic mechanism of periodontitis pregnancy complications. Ann Perriodontol 1998; 3:233-250.

18. Jeffcoat MK, Guers NC, Reddy MS, Cliver SP, Goldenberg RL, Hauth JC. Periodontal infection and preterm birth: results of a prospective study. J Am Dent Assoc 2001; 132:875-880.
19. Silk H, Douglass AB, Douglass JM, Silk L. Oral health during pregnancy. Am Physician 2008; 77:1139-1144.
20. Haake SK, Newman MG, Nisengard RJ, Sanz M: Periodontal microbiology. Clinical Periodontology. Edited by: Newman MG, Takei HH, Carranza FA. 2002, Philadelphia: Saunders, 96-112.
21. Nair PNR: Pathogenesis of apical periodontitis and the causes of endodontic failures. Crit Rev Oral Biol Med. 2004, 15: 348-381.
22. Škrinjarić I. Prevencija oralnih bolesti u djece: U: Bralić I. i sur. Prevencija bolesti u dječjoj dobi. Medicinska naklada. Zagreb, 2014;412-431.
23. World Health Organization. Preterm birth. Fact sheet. Reviewed November 2016. http://www.who.int/mediacentre/factsheets/fs363/en/ (pristup 10.1.2017.),.
24. Mardešić D., Benjak V. Nedonošče. U: Mardešić D. i sur. Pedijatrija. Školska knjiga; Zagreb, 2016;389-397.
25. Crispi F, Bijnens B, Figueras F, Bartrons J, Eixarch E, Le Noble F et al. Fetal growth restriction results in remodeled and less efficient hearts in children. Circulation 2010; 121 (22): 2427-2436.
26. Barker DJ. Human growth and cardiovascular disease. Nestle Nutr Work-shop Ser Pediatr program 2008; 61:21-38.
27. Mitchell RN, Schoen FJ. Atherosclerosis. U: Kumar V, Abbas AK,Fausto N, Aster JC, (ur.). Robbins & Cotran Pathologic Basis of Disease, 8 izd. Philadephia: WB Saunders; 2010;1020-35.
28. Al-Ghamdi A, Jiman-Fatani AA, El-Banna H. Role of Chlamidia pneumoniae, helicobacter pylori and cytomegalovirus in coronary artery disease. Pak J Pharm Sci 2011; 24(2):95-101.
29. Bearfield C, Davenport ES, Sivapathasandarem V, Allaker RP. Possible association between amniotic fluid micro-organism infection and microflora in the mouth. BJOG. 2002;109:527-533.
30. Dunlop AL, Mulle JG, Ferranti EP, Edwards S, Dunn AB, Corwin EJ. The Maternal Microbiome and Pregnancy Outcomes that Impact Infant Health: A Review. Advances in neonatal care: official journal of the National Association of Neonatal Nurses. 2015;15(6):377-385. doi:10.1097/ANC.0000000000000218.
31. Boggess KA, Urlaub DM, Moos MK, Polinkovsky M, El-Khorazaty J, Lorenz C. Knowledge and beliefs regarding oral health among pregnant women. J Am Dent Assoc 2011; 142(11):1275-1282.
32. Glaziou Philippe. Samsize. Computer sample size and power. Version 06.2003. Dostupno s URL: http://sampsize.sourceforge.net/ (pristup 10. 1. 2017).
33. WHO. Child growth standards. WHO Anthro (version 3.2.2., January 2011) http://www.who.int/childgrowth/software/en/
34. World Health Organization. BMI-for - age BOYS. Birth to 5 years (percentiles) Dostupno s URL: http://www.who.int/childgrowth/standards/cht_bfa_boys_p_0_5.pdf?ua=1 (pristup 10. 1. 2017).
35. World Health Organization. BMI-for - age GIRLS . Birth to 5 years (percentiles)
Dostupno s URL: http://www.who.int/childgrowth/standards/cht_bfa_girls_p_0_5.pdf?ua=1 (pristup 10. 1. 2017)

36. Baylor College of medicine. USDA/ARS Children's Nutrition Research Center, Houston, Texas. Age-based Pediatric Blood Pressure Reference Charts. Dostupno s URL: https://www.bcm.edu/bodycomplab/Flashapps/BPVAgeChartpage.html (dostupno 10. 1. 2017).
37. National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents. The Fourth report on the diagnosis, evaluation and treatment of high blood pressure in children and adolescents. Pediatrics 2004; 114 (2 Suppl 4 th Report) 555-576. (dostupno 10. 1. 2017).