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Total Ferric Reducing Ability of Plasma as an independent caries risk marker in pregnant women with diabetes

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Abstract: Pregnancy and diabetes increase the risk of developing pathologies in the oral cavity. Saliva biomarkers can be used as diagnostic markers in the monitoring of dental diseases.
The study aimed to evaluate hard dental tissue health in pregnant women with two forms of diabetes and salivary antioxidant activity to identify optimal non-invasive caries risk factors.
The study involved 104 pregnant women: 35 patients with gestational diabetes mellitus (GDM), 30 patients with type 1 diabetes (T1D), and 39 pregnant patients without diabetes mellitus (control group). Physical examination included caries assessment using the dental DMFT index, caries frequency, SiC index, and dental treatment index. Oral hygiene was also assessed using the Approximal Plaque Index (API). The study of unstimulated saliva measured the total Ferric Reducing Ability of Plasma (FRAP), as well as the activity of superoxide dismutase (SOD), glutathione reductase (GR), and glutathione peroxidase (GPX).
In the analyzed patient population, caries frequency totalled 100%, with active tooth decay observed in 72.12% of patients, whereas caries intensity was similar in the studied groups. In all groups, oral hygiene (API) was average. In T1D group, higher values of the API index ($p = 0.001$) were observed vs. the control group. Antioxidant activity was similar in the study groups. The independent determinants of caries occurrence included API ($p = 0.002$) and FRAP concentration ($p = 0.02$).
The health of dental hard tissues in the examined patients was unsatisfactory and similar in the studied groups. FRAP concentration and oral hygiene (API) functioned as determinants of caries.

Keywords: pregnancy, diabetes, caries, saliva, antioxidants.

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Introduction

Tooth decay is the most common chronic oral disease. It is a multifactorial, dynamic, biofilm-dependent disease, modulated by diet, leading to the loss of minerals from hard dental tissues. It is conditioned by biological, behavioral, psychosocial, and environmental factors and is manifested with demineralization of inorganic substances followed by proteolysis of organic substances in dental hard tissues due to the impact of acids produced by bacteria in the dental plaque as a result of the metabolism of exogenous and endogenous sugars. This process leads to development of carious lesions. [1, 2]

Caries in pregnant women and pregnant women with diabetes is an important healthcare problem, as both pregnancy and diabetes can increase the risk of developing oral disorders. A change of eating habits during pregnancy can affect hard tissues of the teeth, leading to their decay. Usually, carbohydrate intake increases, as well as the frequency of meals and snacking between main meals. This is also accompanied by indigestion and vomiting often occurring in the first trimester, as well as gastroesophageal reflux later in pregnancy. Reduced buffering capacity and pH of the saliva, as well as reduced concentration of mineral compounds also contribute to lesions in dental hard tissues. [3] Sex hormones directly affect the hard tissues of teeth, as it has been shown that odontoblasts and endothelial cells in the pulp feature estrogen receptors. [4] Increase in salivary estrogen levels leads to enhanced proliferation and exfoliation of epithelial cells. This creates better nutritional conditions for bacteria in the oral cavity with an increase in the cariogenic bacteria volumes. Estradiol is metabolized by caries-causing bacteria such as *Streptococcus mutans* and *Streptococcus sanguis.* Higher levels of salivary mucin contribute to the agglutination of these bacteria and enhanced plaque formation. [5]

In type 1 diabetes, higher incidence of caries may be related to such factors as reduced salivary flow rate, increase in salivary glucose, and increase in salivary alpha-amylase associated with both glucose production and adhesion of cariogenic bacteria to the tooth surface. [6, 7] Some authors point to low incidence of caries in patients with type 1 diabetes due to the application of a diabetic diet. It involves absence of sucrose and thus results in reduced glucose production in the oral cavity. [8, 9] No significant progress in carious disease was observed in pregnant patients with gestational diabetes mellitus. [10] Some studies, however, indicate that gestational diabetes mellitus is associated with reduced levels of beneficial oral bacteria, such as Veillonella, which plays an important role in maintaining oral health and preventing tooth decay. Imbalance in the oral microbiome increases the risk of dental diseases, including dental caries, because pathogenic bacteria become more dominant. [11]

Saliva is secreted by the larger salivary glands such as the parotid, submandibular, sublingual, and minor salivary glands. Under physiological conditions in adults, saliva is produced in volumes of about 500–600 ml per day, about half of which is resting saliva, while the rest is stimulated saliva produced in response to various stimuli. Saliva determines correct functioning of the bite apparatus, protects the surface of teeth and the oral mucosa, participates in the perception of taste, temperature and touch, facilitates speech, as well as bite formation and swallowing. The main component of saliva is water, which accounts for about 95–99% of its volume, with the remaining part formed of organic and inorganic components. [12] Saliva is a reservoir of calcium and phosphates, and is saturated with hydroxyapatite, which minimizes demineralization processes on the tooth surface and promotes remineralization. The anti-caries effect of saliva also involves dilution and neutralization of food acids and biofilm bacteria

metabolic products. It also has antioxidative properties, which is of significance in protection against tooth decay. [13]

In the healthy body, oxidative and reducing processes remain in balance. Predominance of oxidation processes leads to an uncontrolled increase in reactive oxygen species due to their overproduction or failure of the body's defense mechanisms. Such a situation is referred to as oxidative stress. This causes damage to many biological structures as well as disruption of processes taking place in the body. Oxidative stress accompanies inflammation and affects its development, also playing a major role in the pathogenesis of many diseases. Oxidative processes are prevented, among others, by antioxidants forming part of the body's immunological mechanisms. The antioxidant properties of saliva ensure balance among free radicals, thus significantly contributing to protecting the oral cavity. Salivary antioxidants include enzymatic and non-enzymatic systems. Glutathione peroxidase, catalase, and superoxide dismutase (SOD) are examples of enzymatic systems, whereas the non-enzymatic antioxidant system includes uric acid and glutathione, together creating the Total Antioxidant Capacity (TAC). [14]

In patients with caries, some biomarkers of oxidative damage were found, as well as changes in the parameters of the antioxidant defense system. [13] Tooth decay is not only a bacterial disease, but it is also host-dependent with factors including the oxidative–reductive balance in the oral cavity. Biomarkers of oxidative damage are elevated in individuals with tooth decay, reflecting an imbalance between free radicals and the antioxidant defense system in the saliva. This can affect the progression of tooth decay by promoting tissue damage and altering local immune responses. [15] There are many salivary molecules changes in concentration or activity of which may prove useful for detecting oral diseases or monitoring response to treatment. Measuring the activity of oxidative stress biomarkers and antioxidants in saliva can potentially serve as a diagnostic or prognostic tool to assess the risk or activity of dental caries. [16]

The present study was aimed to assess the health of dental hard tissues in pregnant women in the context of two different forms of diabetes. Furthermore, the study was to assess the antioxidant activity in saliva in order to determine the optimal, non-invasive factors conditioning the development of caries.

Materials and Methods

The patients were recruited among pregnant women diagnosed with diabetes and treated at the Metabolic Diseases Department of the Jagiellonian University Medical College in Krakow. During the same period, control group sample were recruited among pregnant women without diabetes at the Department of Gynecology and Obstetrics of the Jagiellonian University Medical College. Patients were eligible if they: (1) gave the informed consent; (2) were over 18 years of age; and (3) were in the 3rd trimester of pregnancy. Diagnosis of gestational diabetes mellitus (GDM) or type 1 diabetes (T1D) was made by the attending physician in accordance with the guidelines of the Polish Diabetes Association (Diabetes Poland). The patients were excluded if: (1) they did not give informed consent; (2) they had other types of diabetes mellitus; (3) they were diagnosed with other metabolic diseases affecting carbohydrate metabolism; The study protocol was in compliance with the ethical guidelines of the Helsinki Declaration of 1975 and was approved by the Ethics Committee of the Jagiellonian University Medical College (KBET/270/B/2013). Informed consent was obtained from each patient prior to enrolment in the study.

Medical history was collected from each patient on the basis of their medical records, an oral examination was performed, and saliva samples were collected at the same time. The medical history data collected included information on comorbidities, history of diabetes, current glycemia, family history of diabetes, the presence and type of oral symptoms, and the frequency of visits to the dentist.

A dentist with long experience conducted an oral examination on each patient. The examination was performed using a dental mirror (Pol-Intech, Łódź, Poland) and a single-sided Mifam type dental probe (ZG-1; Pol-Intech, Łódź, Poland). The oral examination included caries and oral hygiene assessment.

Tooth decay was assessed using the DMFT index. It is an indicator of the intensity of the carious process and consists in assessing the components: teeth with decay or temporary filling (D), teeth missing or extracted due to decay (M), teeth filled due to decay (F), and then summing up the individual components.

Caries frequency, i.e., the percentage of people with the DMFT index >0, was also assayed.

Next, SiC index (*Significant Caries Index*) was determined, namely the average DMFT value calculated for 1/3 of patients with the highest DMFT values in the study group.

The dental treatment index was established, expressed as the ratio of the number of filled teeth to the sum of filled teeth and teeth with decay. [17]

Oral hygiene was also evaluated using the Approximal Plaque Index (API). This index was expressed as a percentage and defined as the number of interdental spaces with plaque divided by the number of all assessed spaces. In quadrants I and III, the study was conducted from the lingual side, while in quadrants II and IV, the study was conducted from the buccal side. [18]

Two 1 ml samples of unstimulated saliva were collected from each patient. Sampling was performed between 8:00 a.m. and 11:00 a.m., at least 1 hour after the last meal and before the dental examination. Calibrated tubes were filled with saliva flowing freely from the mouth. The samples were immediately centrifuged at 10,000 g for 5 minutes at 4°C, then frozen and stored at −80°C until the end of the sampling period; they were thawed immediately prior to testing.

The analysis involved an assessment of Total Antioxidant Capacity (TAC), extracellular SOD activity, GR activity, and GPX activity. SOD activity was measured using the Misra and Fridovich method. [19] Total Antioxidant Capacity was measured using the Benzie and Strain Ferric Reduction Ability of Plasma (FRAP) method. [20] A modified Goldberg method was used to measure GR activity. [21] GPX activity was measured using the method described by Paglia and Valentine. [22] Antioxidant analysis was performed using the ELx808 Absorbance Microplate Reader (BioTek Instruments). The measurements were conducted at the Department of Diagnostics, Department of Clinical Biochemistry, Jagiellonian University Medical College in Krakow.

Statistical analysis was conducted using the STATISTICA PL data analysis software, version 9.1 (StatSoft Polska, Kraków, Poland), and the MedCalc® software, version 8.1.1.0 (https://www.medcalc.org/). Continuous variables have been presented as median and interquartile range (IQR), while categorical variables as number and percentage (n,%). The parameters were compared between patients with gestational diabetes mellitus, type 1 diabetes, and the control group. The Kruskal-Wallis test and post-hoc analysis were applied to compare continuous variables. For dichotomous variables, the χ^2 test was used. The stepwise logistic regression model was applied to assess the factors determining tooth decay. The accounted variables featured p-value <0.20. The results have been presented with odds ratios (OR) and confidence intervals (CI). The significance level was set at $p < 0.05$.

Results

Group description

The study involved 104 pregnant patients, including 35 patients with gestational diabetes mellitus (GDM), 30 patients with type 1 diabetes (T1D), and 39 pregnant patients without diabetes mellitus (control group). Pregnant women in each group did not differ in terms of age, height, pre-pregnancy body weight, pre-pregnancy body mass index (BMI), body weight during pregnancy, incidence of GDM in a previous pregnancy, and diabetes in the family. Hypothyroidism and Hashimoto's thyroiditis were more frequent in patients with T1D vs. patients with GDM and the control group (11 (36.7%) vs. 4 (11.4%), respectively, p = 0.0200; 11 (36.7%) vs. 6 (15.4%), p = 0.0001; and 10 (33.3%) vs. 1 (2.9%), p = 0.0010; 10 (33.3%) vs. 0 (0%), p = 0.0400). No differences were recorded among the groups regarding incidence of other diseases. Fasting plasma glucose (FPG, mmol/L) was the highest in T1D patients, lower in GDM patients, and lowest in the control group (6.22 (5.16–7.55) vs. 4.77 (4.50–5.16) vs. 4.34 (4.14–4.82), respectively; p <0.0001). HbA1c hemoglobin level in T1D patients amounted to 5.75% (5.10–6.10%). Detailed data have been presented in Table 1.

Table 1. Group data upon enrolment in the study.

Variable	Total	Control group	GDM	T1D	p
Number [n]	104	39	35	30	
Anthropometric data					
Age [years]	31.0 (28.0–33.0)	30.0 (26.0–33.0)	32.0 (29.0–34.0)	31.0 (28.0–33.0)	0.39
Height [cm]	165.0 (161.0–170.0)	165.0 (162.0–169.0)	164,0 (160.0–168.0)	167.5 (163.0–170.0)	0.12
Body weight before pregnancy [kg]	63.0 (56.5–69.0)	62.0 (57.0–65.0)	65.0 (56.0–74.0)	61.5 (54.5–73.0)	0.49
BMI before pregnancy [kg/m^2]	22.7 (21.6–25.2)	22.8 (21.8–23.6)	23.8 (21.6–27.5)	22.2 (20.8–25.9)	0.17
Body weight during pregnancy [kg]	73.6 (68.0–82.2)	73.6 (69.6–80.5)	74.5 (65.5–85.5)	73.5 (68.0–85.8)	0.90
Examination					
Prior gestational diabetes mellitus n (%)	8 (7.7)	2 (5.1)	5 (14.3)	1 (3.3)	0.19
Prevalence of diabetes in family n (%)	50 (48.2)	16 (41.0)	18 (51.4)	16 (53.3)	0.53
Hypothyroidism n (%)	21 (20.2)	6 (15.4)*	4 (11.4)†	11 (36.7)*	**0.03**
Hashimoto' disease n (%)	11 (10.6)	0 (0)*	1 (2.9)†	10 (33.3)*	**0.0001**

Table 1. Cont.

Variable	Total	Control group	GDM	T1D	p
Hypothyroidism diagnosed during pregnancy n (%)	6 (5.8)	0 (0)	2 (5.7)	4 (13.3)	0.06
Hypertension n (%)	3 (2.9)	1 (2.6)	2 (5.7)	0 (0)	0.39
Hypertension diagnosed during pregnancy n (%)	7 (6.7)	3 (7.7)	3 (8.6)	1 (3.3)	0.67
Intrahepatic cholestasis of pregnancy n (%)	3 (2.9)	3 (7.7)	0 (0)	0 (0)	0.09
Migraine n (%)	1 (1)	0 (0)	1 (2.9)	0 (0)	0.37
Laboratory tests					
Fasting plasma glucose level [mmol/l]	4.82 (4.32–5.39)	4.34 (4.14–4.82)	4.77 (4.5–5.16)*†	6.22 (5.16–7.55)*	**<0.0001**

* p <0.05 vs. control group.
† p <0.05 between the groups of patients with type 1 diabetes and gestational diabetes mellitus.
Abbreviations used in the table: BMI — body mass index, T1D — type 1 diabetes, GDM — gestational diabetes mellitus, p — test probability.

Dental history

The most common oral ailment reported by patients during pregnancy was increased gingival bleeding, both spontaneous and provoked. It occurred with equal frequency in the study groups (p = 0.31) and concerned a total of 50 patients (48.1%), including 19 (54.3%) with GDM, 16 (53.3%) with T1D, and 15 (38.5%) from the control group. Other complaints included tooth and gum hypersensitivity, which affected a total of 14 (13.5%) patients, and bad breath reported by 2 (1.9%) pregnant women. The incidence of reported complaints was similar in all groups. The results have been presented in Table 2.

The frequency of visits to the dental office was similar in all groups (p = 0.44). Most pregnant women reported that they visit regularly, more than once per year. There were 23 such patients in the GDM group (65.71%), 13 in the T1D group (43.33%), and 21 such patients in the control group (53.85%). Also, a large number of pregnant women visited dentist once per year.

Assessment of tooth decay and oral hygiene

When assessing tooth decay, it was observed that the occurrence of caries, teeth extracted due to tooth decay, or the presence or absence of fillings were similar in all the study groups. The active tooth decay process occurred in 75 patients (72.12%), whereas 29 (27.88%) of them were free of

Table 2. Dental history data.

Variable	Total	Control group	GDM	T1D	p
Increased gingival bleeding n (%)	50 (48.1)	15 (38.5)	19 (54.3)	16 (53.3)	0.31
Tooth and gum hypersensitivity n (%)	14 (13.5)	6 (15.4)	3 (8.6)	5 (16.7)	0.58
Bad breath n (%)	2 (1.9)	1 (2.6)	0 (0.0)	1 (3.3)	0.58

Abbreviations used in the table: T1D — type 1 diabetes, GDM — gestational diabetes mellitus, p — test probability.

caries. In the control group, caries-related cavities were observed in 28 (71.79%) patients vs. 27 (77.14%) in the GDM group vs. 20 (66.66%) in the T1D group. Teeth extracted due to decay were recorded in exactly half of pregnant women: 52 (50%). There were 18 (46.15%) such patients in the control group, 19 (54.29%) in the GDM group, and 15 (50%) in the T1D group. Fillings were present in 100 (96.15%) patients, with only 4 women (3.85%) never treated conservatively for caries. In the control group, fillings were found in 38 (97.44%) patients vs. 34 (97.14%) the GDM group vs. 28 (93.33%) in the T1D group.

The average number of teeth with decay (D), missing/extracted due to decay (M) and filled (F) did not differ significantly among groups ($p = 0.11$; $p = 0.78$; $p = 0.06$, respectively). The minimum and maximum D values were 0 and 16; M values: 0 and 14, and F values: 0 and 20, respectively.

Caries frequency in the studied patient population totalled 100%, which indicates that all pregnant women examined were previously or currently affected by tooth decay. The mean DMFT score among the participants (caries intensity) was calculated as 15.1 (SD = 5.02) and ranged from 4 to 30. For the control group, mean DMFT value was 14.8 (SD = 4.0.9) vs. 14.1 (SD = 4.84) in the GDM group vs. 16.7 (SD = 6.0) in the T1D group. The median of DMFT values for the entire study group was set at 15 (11.5–18), whereas median DMFT values in individual groups were as follows: 14 (10–19) for GDM, 17 (12–22) for T1D, and 16 (12–18) for the control group. Statistical analysis did not reveal any significant differences among the groups ($p = 0.1$).

The SiC index (Significant Caries Index), being the mean DMFT value in 1/3 of patients with the highest values of the index, totalled 17 for the entire population. SiC values in individual groups were similar: 17 in the control group, 16 in the GDM group, and 18 in the T1D group. The mean caries treatment index amounted to 0.86 (range: 0.57–1.00).

In pregnant women with T1D, higher values of the API index ($p = 0.001$) were observed vs. the control group. In the group of patients with GDM, there were no statistically significant differences in the oral hygiene compared to the control group. In all groups, the mean API index value pointed to average oral hygiene.

Index values in individual study groups have been presented in Table 3.

When analyzing the prevalence of caries in individual groups of patients, a positive correlation was found between the advancement of caries measured with the D value and the HbA1c level ($R = 0.41$, $p = 0.02$). Because the HbA1c assay was available for patients with T1D, the correlation was found only in this group (Fig. 1). In the case of M, F, and DMFT values, no such correlation has been evidenced.

Table 3. Tooth decay and oral hygiene assessment in the studied population of pregnant patients.

Variable	Total	Control group	GDM	T1D	p
D	2 (0-5)	2 (0-5)	3 (1-6)	2 (0-4)	0.11
M	0.5 (0-2.5)	0 (0-2)	1 (0-3)	0.5 (0-3)	0.78
F	11.5 (7-14)	12 (7-15)	8 (6-12)	12.5 (7-16)	0.06
DMFT	15 (11.5-18)	16 (12-18)	14 (10-19)	17 (12-22)	0.1
Caries intensity	15.1	14.8	14.1	16.7	0.1
Caries dental treatment index	0.86 (0.57-1)	0.88 (0.58-1)	0.75 (0.46-0.93)	0.87 (0.71-1)	0.64
API (%)	50 (38-79)	46 (31–64)	50 (39-66)	69 (44-97)*	**0.03**

*p <0.05 vs. control group (values given in the text)
Abbreviations used in the table: T1D — type 1 diabetes, GDM — gestational diabetes mellitus, D — number of teeth with decay, p — test probability, M — number of teeth extracted due to decay, F — number of teeth filled due to decay, DMFT — caries intensity index.

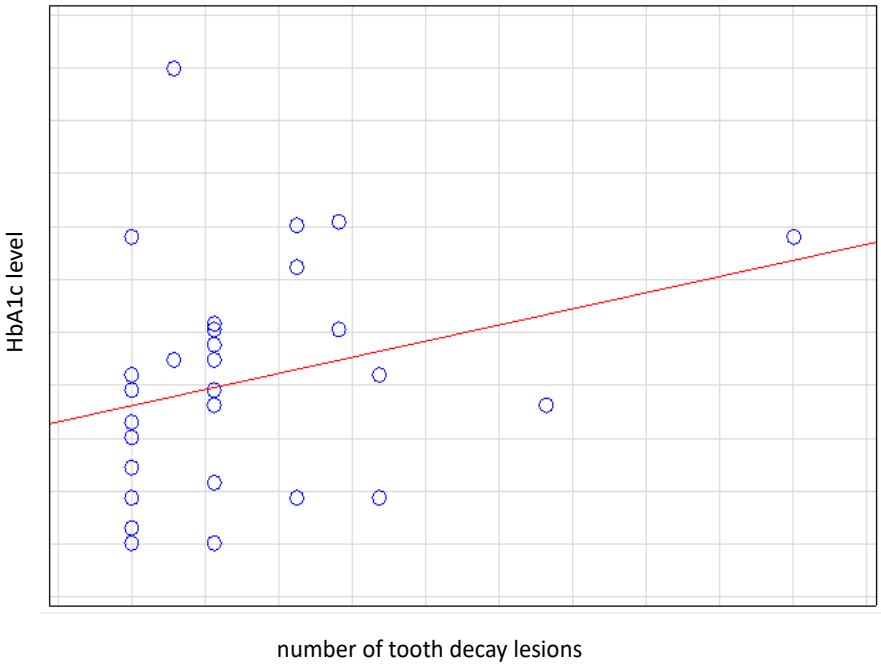

Fig. 1. Correlation between the number of carious cavities D (X axis) and the HbA1c level (Y axis) in patients with Type 1 DM.
X axis — number of tooth decay lesions
Y axis — HbA1c level
Line — correlation curve

Selected antioxidant levels in non-stimulated saliva

In all patients, total ferric reducing ability of plasma (FRAP) and the activity of extracellular superoxide dismutase (SOD), glutathione peroxidase (GP), and glutathione reductase (GR) were assayed.

In the groups studied, the above-mentioned parameters remained at a similar level. Detailed concentrations of salivary antioxidants assayed have been presented in Table 4.

Table 4. Selected salivary antioxidants levels in the study groups.

Variable	Total	Control group	GDM	T1D	p
FRAP [mmol/l]	0.42 (0.32–0.53)	0.41 (0.33–0.55)	0.42 (0.3–0.51)	0.43 (0.28–0.56)	0.97
Glutathione reductase [U/l]	9.85 (5.4–14.2)	11.5 (5.7–16.4)	10.3 (6.2–15.8)	7.9 (4.7–10.7)	0.22
Glutathione peroxidase [U/ml]	29.25 (14.5–71.35)	37.2 (15.1–67)	25.8 (11.7–71.0)	27.1 (19.1–96.3)	0.78
Superoxide dismutase [U/ml]	12.8 (10.65–15.7)	14.1 (10.9–17.7)	12.5 (9.5–14.2)	12.55 (10.6–15.3)	0.38

Abbreviations used in the table: T1D — type 1 diabetes, FRAP — Ferric Reducing Ability of Plasma, GDM — gestational diabetes mellitus, p — test probability.

The assessment of factors determining the occurrence of tooth decay was performed using the (stepwise) logistic regression models. When selecting determinants, variables accounted for included oral hygiene (measured with API), selected salivary antioxidant levels (FRAP, dismutase, peroxidase, reductase), diagnosed diabetes, hypothyroidism, and the patient's age. In the regression model, variables with $p < 0.2$ were taken into account in the subsequent steps. Variables with $p \geq 0.2$ were excluded from the model. Variables with $p < 0.05$ were considered significant determinants.

In the logistic regression model ($p < 0.0001$), the determinants of caries included API (OR = 1.03; 95% CI: 1.01–1.06; p = 0.002) and FRAP concentration (OR = 33.2; 95% CI: 1.74– 637.0; p = 0.02). Each increase in FRAP concentration by 1 mmol/L increases the odds of developing caries by 33 times, regardless of oral hygiene (an increase in API by 1% independently increases these odds by 3%). The results have been presented in Table 5.

Table 5. Logistic regression model.

Variable	OR	95% CI	p
API	1.03	1.01–1.06	**0.002**
FRAP	33.2	1.74–637.0	**0.02**

Abbreviations used in the table: API — Approximal Plaque Index, FRAP — Ferric Reducing Ability of Plasma, OR — odds ratio, p — test probability, CI — confidence interval.

Discussion

In the study, patients did not differ in the incidence of oral complaints or the frequency of visits to the dentist. The main reported complaints involved gingival bleeding and hypersensitivity of teeth and gums. An imbalance between the bacterial plaque activity and the body's defense reactions in pregnant women can lead to gestational gingivitis, manifested with redness, hyperplasia, and swelling of the papillae and marginal gingiva with associated bleeding while brushing. [23] Also, according to some authors, gingivitis may be more frequent and more severe in patients with type 1 diabetes. The intensity of periodontal lesions is associated with the duration of diabetes. [24] Most patients reported regular visits at the dental office once per year or more frequently. The patients declared that, since they became pregnant, the frequency of visits increased. This may be related to the growing patient awareness of the need to take care of their own health in such a special period as pregnancy, as well as the position of the Working Group on Dental Prevention in Pregnant Women of the Polish Chapter of the Alliance for a Cavity-Free Future, which recommends at least two dental consultations during pregnancy. [25, 26]

Active tooth decay process occurred in 72.12% of patients with caries frequency hitting 100%, which points to high prevalence of caries in the studied population. The values of the analyzed tooth decay indicators (D, M, F, DMFT, SiC, and treatment index) were comparable among the study groups, which indicates a similar level of tooth decay occurrence and severity regardless of GDM or T1D. Also López-Pérez *et al.*, when examining pregnant patients with type 2 diabetes, gestational diabetes mellitus, and those without diabetes, evidenced that they recorded a similar caries incidence with DMFT in all examined patients higher than 0. [10] In the conducted study, the highest values in all groups referred to the F numbers, which points to an intensive history of tooth decay, but also effective conservative treatment and control of caries lesions. Also, the values of the treatment index in the study groups point to a high level of benefiting from dental care and good availability of the services. Mean values of D — teeth with tooth decay, and especially M — teeth missing due to tooth decay achieved much lower values. The average D = 2 number indicates that the current disease activity is relatively high and requires dental intervention, particularly during pregnancy, or with additional complication of pregnancy with diabetes. In the studies by other authors, similar D-values were obtained, amounting to about 1.2–1.7 in pregnant women, which reflects a temporary increase in the risk of caries in the course of pregnancy, but at the same time suggests that, in most patients, the disease is controlled in a way owing to prior treatment and maintained access to dental care. [27, 28]

The study found that the incidence of tooth decay was higher in patients with higher HbA1c levels ($r = 0.41$, $p = 0.02$). Because the HbA1c assay was available for patients with T1D, the correlation was found exclusively in this group. HbA1c is not titrated in pregnant women without diabetes. There is also no evidence of HbA1c being useful as a metabolic control monitoring tool in GDM. [29]

Several studies have shown that a higher incidence of caries was observed in adolescents with diabetes and higher HbA1c values compared to those with good metabolic control. This is especially true for patients with persistently elevated values of this parameter, i.e., with poorly controlled diabetes. On the one hand, a decrease in the new tooth decay can be observed in the years following the diagnosis of the disease with appropriate metabolic control, but the long duration of the disease may contribute to an increase in caries intensity. [30, 31] According to a study by ElBshari *et al.*, diabetes and poor glycemic control have either no effect on the risk of caries, or the

impact is so low that it is masked by other known and more dominant risk factors, such as diet and obesity. [32] Also, in the studies by Tan *et al.*, no significant correlations were found between the risk of tooth decay and HbA1c levels. [33] However, the above-mentioned studies were conducted in children or adolescents, and were based on the overall DMFT index, not only on the number of teeth with caries.

In all groups, the mean API index value pointed to average oral hygiene. In pregnant women with T1D, higher API values were observed vs. the control group. Pregnant women may experience a temporary deterioration in oral hygiene. This is associated with increased frequency of eating, frequent fatigue, nausea, vomiting and, in the final stage of pregnancy, also reflux, as well as greater susceptibility to gingivitis. [3]

In the study, the salivary antioxidant levels were similar in all groups. In diabetes, oxidative stress is mainly observed due to poor glycemic control, so it is likely that patients in the gestational diabetes mellitus and type 1 diabetes groups had diabetes under proper control. The analysis of the literature indicates a lack of clarity in the previous reports, which is related to the evident discrepancy of the reported results. In studies comparing antioxidant levels in patients with gestational diabetes mellitus vs. pregnant women without diabetes, it was noted that the group with GDM did not show a significant difference in the total antioxidant capacity of saliva whereas all other antioxidant markers decreased compared to the healthy control group. [34] The literature also provides information that, in similar studies, the total antioxidant capacity of saliva increased [35] or decreased [36] in pregnant women with gestational diabetes mellitus compared to non-diabetic women. Discrepancies may result from differences in the methods of laboratory testing used and in the trimesters of pregnancy in which the tests were conducted.

Multifactorial analysis revealed that the variable determining the occurrence of tooth decay is the total Ferric Reducing Ability of Plasma (FRAP). Similar results were obtained by other authors. In their studies, Hegde *et al.* showed that, in adults, an increase in total antioxidant capacity in saliva and plasma correlated with the DMFT value increase. The same author in other studies also evidenced an increase in total antioxidant capacity in patients with tooth decay in relation to the caries-free control group. [37, 38] Uberos *et al.* showed in their study that, in patients with caries, total antioxidant capacity in saliva was 2.89 times greater than in patients without caries. [39] Similar results were also obtained in studies involving children and adolescents. [40, 41, 42] The authors used different methods to determine total antioxidant capacity, with Shaki, Silva and Mahjoub using the FRAP method, which has also been used in current research. Regardless of the method applied, the results were similar. Oxidative stress can initiate and participate in the progression of many inflammatory and infectious diseases, such as caries. The development of caries correlates with changes in salivary oxidative biochemistry, including an increase in lipid peroxidation and total antioxidant capacity in chronic carious lesions. After treatment of carious lesions, however, a decrease in lipid peroxidation is observed. Saliva may be the first line of defense against harmful effects of oxidative stress in oral diseases. The increase in total antioxidant capacity in saliva in people with tooth decay may point to a compensatory mechanism against free radicals. [40, 43] In the study conducted, the activity of other antioxidants did not affect caries occurrence. According to some authors, the activity of superoxide dismutase was increased in patients with caries compared to the control group without caries. [44, 45] The proposed multivariate regression model did not, however, confirm these reports.

Tooth decay was also affected by oral hygiene measured with API. This is not a surprising result, as the presence of plaque is a documented factor involved in the development of caries.

Dental biofilm determines the initiation and progression of demineralization lesions. In the oral cavity, especially in conditions of impaired hygiene, excessive growth of cariogenic bacteria occurs with predominance of *Streptococcus mutans*, *Streptococcus sobrinus*, as well as acidophilic and acid-forming bacteria such as *Lactobacillus spp*. The bacterial biofilm metabolizes carbohydrates, leading to the formation of organic acids which initiate the process of enamel demineralization through calcium ions and phosphates being washed out of its structure. Prolonged persistence of biofilm in an acidic environment shifts the balance towards more acid-forming bacteria and reduces the ability of saliva to neutralize acids. [46]

The effect of diabetes on the presence of caries has not been demonstrated. Gestational diabetes mellitus is one of the most common metabolic complications of pregnancy. [47] According to the data from the literature, no increased tooth decay intensity was observed in pregnant patients with gestational diabetes mellitus vs. healthy pregnant patients. [10] According to some reports regarding type 1 diabetes, high concentrations of glucose in the saliva and secretions of the gingival pockets, as well as reduced salivation, lower pH, and increased density, as well as frequent meals contribute to the development of tooth decay in patients. [48, 49] Other reports indicate that dietary restrictions excluding simple carbohydrates lead to lower incidence of caries in such patients. [50] Caries intensity may also depend on HbA1c levels. The regression model did not account for HbA1c level because the data were available exclusively for 30 patients with type 1 diabetes, which would limit the possibility of conducting the analysis.

All the examined patients were of a similar age, so it is not surprising that age was not decisive for the incidence of caries.

Hypothyroidism did not affect caries incidence in the study population either. Reports from the literature are unclear. Venkatesh *et al*. showed that, in children with hypothyroidism, slightly higher values of the DMFT index were observed but these differences were not statistically significant. [51] Some studies conducted on extracted teeth in patients with hypothyroidism point to structural changes in them within the enamel, dentin, and cementum, both healthy and decayed teeth. [52]

The conducted study has some limitations. One of these involves the inability to perform tooth decay assay on the basis of dental x-ray imaging. X-rays during pregnancy are subject to medical restrictions, except in the most urgent cases. Another limitation is that only a few saliva biomarkers were included in the study, which indicates the need for further research.

The health of hard dental tissues in the examined patients was unsatisfactory and similar in the studied groups. No correlation was found between FRAP and caries in pregnancy subgroups. The study showed that the total Ferric Reducing Ability of Plasma (FRAP) and oral hygiene (API) functioned as determinants of caries.

Authors' contributions

J.W. collected, interpreted the data and was a major contributor in writing the manuscript. B.K.-C., D.P.-G., K.G. were responsible for laboratory analyses. J.P.-P. was responsible for the final version of the manuscript and approved it for submission. All authors read and approved the final manuscript.

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Conflict of interest

None declared.

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