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Interleukin 6 in pregnancy with gdm

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Abstract
Background: Gestational diabetes melitus is a glucose intolerance diagnosed for the first time in pregnancy which may lead to maternal, fetal and neonatal unfavourable outcome. In GDM inflammatory markers like interleukin 6 are elevated and may provide informations on pathophysiology and prediction of perinatal risk.
Aim: to evaluate the average concentration of interleukin 6 in GDM and normoglycemic women and the influence of body mass index on concentration of IL-6 in these women.
Material and Methods: A case control study was made at the University Clinic for obstetrics and gynecology, Skopje in a period of one year. 100 pregnant women were selected from the pregnant women that performed 75g OGTT in the second trimester for sreening for GDM. Body mass index was calculated according to the terms of Institute of medicine and pregnant women were divided in 4 groups: GDM, BMI>25 (n=25); GDM, BMI<25(n=25); normoglycemic, BMI>25(n=25); normoglycemic BMI<25(n=25). Serum levels of IL-6 were analysed with ELISA method.
Results: The medium values of IL-6 were higher in GDM compared to controls (2.77 ± 1.1 pg/ml vs 2.16 ± 0.5 pg/ml, p=0.0016).
Pregnant women with GDM and BMI>25 vs GDM and BMI < 25 had statistically different values of IL-6 (2.58 ± 1.1 vs 2.19 ± 0.5 pg/ml, p=0.0019). Overweight women with GDM had significantly higher interleukin 6 than overweight women without GDM (3.06 ± 1.4 pg/ml vs 2.28 ± 0.7 pg/ml, p=0.021).
Average value of IL-6 in GDM women with normal weight was 2.48 ± 0.8 pg/ml and significantly higher than normoglycemic women with normal weight, 2.04 ± 0.1 pg/ml, p=0.016. IL- 6 had insignificantly higher values in the overweight women from the control group compared to normal weight women from the control group (2.28 ± 0.7 pg/ml vs 2.04 ± 0.1 pg/ml; p=0.11).
Conclusion: IL-6 is significantly higher in GDM compared to normoglycemic women. It can be used in addition with other biomarkers in eventual prediction of this condition.

Keywords: GDM, normoglycemic women, BMI, IL-6

1. Introduction

1.1 Inflamation in pregnancy

Pregnancy itself is accompanied with altered inflammation profile compared to nongravid state. Nonlimphoid tissue including placenta especially trophoblastic cells are main place of cytokine production in pregnancy. During normal pregnancy balance between T-helper cellular activity is highly changed towards antiinflamatory profile and characterized with Th-2 cytokines that have a protective part in feto-maternal connection and favourise normal outcome of pregnancy [1].
Inhanced inflammatory response with adipokines locally (adipose tissue, placenta and vascular endothelium) and systemically (circulating plasmatic concentrations) may be part of the infavourable outcome of pregnancy. In the last years clinical and epidemiological studies have shown clear connection between inflammatory parametars and methabolic diseases like obesity, type 2 diabetes and GDM [2].

1.2. Biomarkers in GDM

Subject of interest of numerous studies are serum markers like inflammatory, adipokines, endothelial functional markers and lipid methabolism markers in GDM.
More authors emphasise the potential use of these markers in prediction of GDM and predictive models for early intervention. [3].

1.3. Interleukin-6 (IL-6)

Interleukin 6 (IL-6) acts as proinflammatory cytokine and antiinflamatory myokine. In the human population it is encoded by IL6 gene and is secreted by T cells and macrophages which stimulate the immune response during infection and trauma [4]. IL6 is widely spread in female reproductive tract and gestational tissues and has regulatory function in embryonal implantation and placental development as well as immune adaptation and tolerance of pregnancy (picture 1). Altered cytokine profile

occurs in idiopatic infertility, recurrent spontaneous abortions, preeclampsia and preterm deliveries [4].

Cutt off values of IL-6 in the general population are between 0.31 and 5.00 pg/mL. Althow frequently investigated in pregnancies there are no defined reference range values of IL-6 in the literature in the 3 trimesters of pregnancy. According to study of Graig *et al* medium value of interleukin 6 in the second trimester was 1.9 pg/mL, in preterm labour 9.3 pg/mL, in chorioamnionits 15.9 pg/mL. In term pregnancy medium value of IL6 was 2.2 pg/mL and in term delivery 4.7 pg/mL [3].

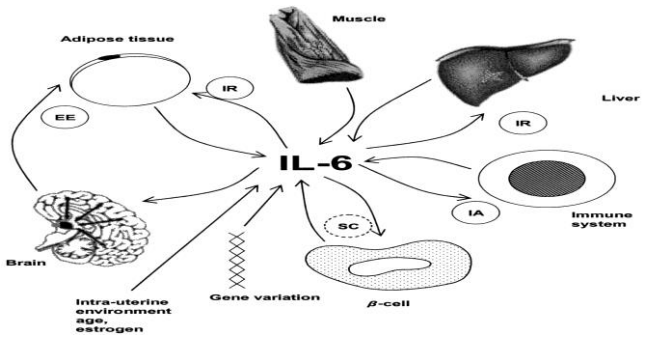

Fig 1: IL6 and pregnancy

1.4. Interleukin 6 in gestational diabetes mellitus

Increase of IL-6 in pregnancy is due to placental production. In obesity, inflammation, GDM, type 2 diabetes there is an increase of circulating levels of IL6 two or three times over the referent values. Increase of IL-6 has a positive correlation with increase of glucose, body mass index and reduced insulin sensitivity.

Yi *et al* confirmed that serum levels of IL-6 and high sensitive CRP are elevated in GDM and through various mechanisms take part in pathogenesis of GDM [4].

Morisset, Dube *et al* made a longitudinal study of 47 women that were screened with 75g OGTT (20 GDM, 27 controls). IL-6 was measured with ELISA during GDM screening and 2 months postpartum. IL-6 concentrations were significantly higher in GDM compared with controls (1.47 ± 0.72 vs. 0.90 ± 0.32 pg/mL). Similar results came from the study of postpartal period, IL-6 was significantly higher in GDM compared to controls (1.88 ± 0.85 vs. 1.41 ± 0.87 pg/mL) [5]. Results showed that GDM is associated with elevated IL-6 levels irrespective of the degree of obesity during pregnancy and after birth.

IL-6 stimulates the production of acute fase proteins in reply to various stimules.

Houser *et al* made a study of 1600 women from which 5% developed GDM and confirmed that elevated levels of IL-6 are associated with increased likelihood of GDM development. The rate of GDM was significantly higher in the group of elevated IL-6 compared with the normal group, 10.5% vs 4.9% [6]. Nergiz *et al* published that galanin and IL-6 are significantly associated with the markers of insulin resistance and may play an importrant role in regulation of glucose homeostasis [7].

Hassiakos *et al* made a study of maternal serum concentration of IL-6 in 11-14 gw in normal and pregnancies complicated by GDM. IL-6 was elevated in GDM group (medium concentration 2 pg/ml) compared with control group (medium 1.5 pg/ml). According to this study combination of maternal characteristics and serum levels of IL-6 may provide a predictive model for GDM [8].

Further studies for role of inflammatory mediators in GDM pregnancies in every trimester of pregnancy and postpartal period are needed.

2. Aim

To establish:

-Medium value of IL-6 in GDM and normoglycemic women;

-The connection between higher value of IL-6 and GDM;

-Influence between obesity in pregnancy with GDM on the levels of this biomarker;

-Influence of BMI on levels of IL-6 in normoglycemic women;

-Informations that would be helpful in clinical practice.

3. Material and methods

A case control study was performed at the University Clinic for gynecology and obstetrics in Skopje in the period of 2016 and 2017. One hundred pregnant women which attended the outpatient department for prenatal care at the clinic in that period were included in the study.

Body mass index was calculated by a medical scale (kg/m2) according to the guidelines of Institute of medicine [9] and pregnant women were classified as:

- normal weight: BMI =18.5-24.9,
- -overweight: BMI=25-29.9 and
- -obese: BMI >30.

From the pregnant women that performed 75g oral glucose tolerance test (OGTT) at the biochemical laboratory in the Clinic, women with GDM and normal OGTT were selected at the same gestational age, parity and maternal age. Pregnant women were refered for OGTT from the ob@gyn specialist from primary, secondary or tertiary level of care for performing universal screening for gestational diabetes melitus.

All patients were divided in 4 groups:

1. Normal OGTT and BMI<25kg/m^2 (n=25);
2. Normal OGTT and BMI>25kg/m^2 (n=25);
3. GDM and BMI <25kg/m^2 (n=25) and
4. GDM and BMI>25kg/m^2 (n=25).

Pregnant women were interviewed by a doctor with standard questionare written on Macedonian language.

3.1. Criteria for participation in the clinical study

Inclusion criteria were:maternal age 18-45 years, eligibility of the pregnant women for follow up, gestational age confirmed with first trimester ultrasound, first prenatal control performed before 20 gw, GDM diagnosed with 75g OGTT according to the criteria of the IADPSG [10].

Pregnant women with: pregestational diabetes, chronic hypertension, chronic inflammatory bowel disease treated by corticosteroids, stillbirth, fetal anomalies and amnioinfectious syndrome were excluded.

3.2. Diagnosis of GDM

GDM was diagnosed according to the recomendations of the IADPSG [10] between 24 and 28 gestational week with 75g oral glucose tolerance test (glucose oxydase, Beckman Glucose Analyzer) performed in the morning after night feast with venous blood drawn 60 and 120 minutes after ingestion of 75 g glucose dissolved in 200ml water with cut off values: 0' < 5,1; after 1-h < 10,0; after 2-h < 8,5 mmol/L. Gestational age was calculated by date of last period and confirmed by first trimester ultrasound. IL-6 was analysed from peripheral blood taken from the cubital vein. The serum was obtained by centrifugition (1000xg in the period of 20 minutes) and frozen at -20C for additional analyses after collection of all samples. In a certified laboratory (certificate MKC EN ISO 15189 - 2013) complete analyses were performed.

3.3. Principes of the test IMMULITE 1000

IL-6 is a test for in vitro diagnostic use and quantitative measurement of IL-6 in serum, EDTA or heparinized plasma as a help in investigation of inflammatory processes. IMMULITE 1000 IL-6 is an enzyme marked, hemiluminiscent, sequentional immunometric assay. IL-6 test units contain a seat marked witn monoclonal anti IL-6 antibody. Expected values – nonparametric low 95% values are from non-detectable to 3.4 pg/ml. Apsolute value is from nondetectable till 5.9 pg/ml. Analytical sensitivity is 2 pg/ml [11].

4. Statistics

The statistics is made by computer program SPSS 23 for Windows.

- -numeric values are given with descriptive statistics (arithmetic median, standard deviation, mediana and interquartal interval);
- -qualitative values are shown with apsolute and relative;
- -for comparison of analysed groups independent parametric and nonparametric tests were used (Student t-test for independent samples, Mann-Whitney test);
- -for all analyses p value < 0,05 was taken for statistical significance.

5. Results

5.1. Medium values of IL-6 in GDM vs normoglycemic women

IL-6 had higher medium values in GDM women compared to pregnant women with negative OGTT (2.77 ± 1.1 pg/ml vs 2.16 ± 0.5 pg/ml). The difference of 0.61 between the 2 groups was statisticaly confirmed as significant for p=0.0016, (table 1).

Table 1: Average values of IL-6 in GDM and control group

Groups	Descriptive Statistics (IL-6 pg/ml)			p value
	mean ± SD	std. err.	min-max	
GDM	2.77 ± 1.1	0.17	2 – 7.6	t=3.2 p=0.0016 sig
Control Group	2.16 ± 0.5	0.07	2 – 2.08	

t (Student t-test)

5.2. IL-6 in GDM, BMI>25 vs GDM, BMI<25

Pregnant women with GDM and BMI>25 vs GDM, BMI<25 had statistically significantly different values of IL-6 (p=0.0019) with higher values in women with GDM and BMI>25.

Average values of IL-6 in overweight pregnant women with GDM compared to normal weight women was 2.58 ± 1.1 and 2.19 ± 0.5 pg/ml concequently, (table 2).

Table 2: Average values of IL-6 in GDM, BMI > 25 vs GDM, BMI < 25

GDM group				
Groups	Descriptive Statistics (IL-6 pg/ml)			p value
	mean ± SD	std err	min – max	
Overweight	2.58 ± 1.1	0.161	2 – 7.6	t =2.27 p=0.025 sig
Normal weight	2.19 ± 0.5	0.075	2 – 5.24	

t (Student t-test)

5.3. Comparrison of interleukin 6 in GDM, BMI>25 vs control group BMI>25

Overweight pregnant women with or without GDM had significantly different values of interleukin 6. For a value of p=0.021, statisticaly significant difference was found between overweight pregnant women with or without GDM about the value of interleukin 6.

IL-6 values were significantly higher in overweight pregnant women with GDM compared to overweight pregnant women without GDM (3.06 ± 1.4 pg/ml vs 2.28 ± 0.7 pg/ml; difference 0.78, table 3).

Table 3: IL-6 in GDM, BMI > 25 vs control group, BMI > 25

GDM (BMI > 25) / control group (BMI > 25)				
Groups	Descriptive Statistics (IL-6 pg/ml)			p value
	mean ± SD	std err	min – max	
GDM, BMI>25	3.06 ± 1.4	0.2	2 – 7.6	t =2.39 p=0.021 sig
Control group, BMI > 25	2.28 ± 0.7	0.1	2 – 5.24	

t (Student t-test)

5.4. Comparrison of IL-6 between GDM, BMI<25 vs control group, BMI<25

IL-6 was significantly different between GDM women with normal weight vs control group with normal weight.

Pregnant women with GDM and normal weight had significantly higher IL-6 than normal weight pregnant women from the control group (2.48 ± 0.8 pg/ml vs 2.04 ± 0.1 pg/ml, p=0.016, table 4).

Table 4: Average value of IL-6 in GDM, BMI < 25 vs control group and BMI <25

GDM (BMI < 25) / control group (BMI < 25)				
Groups	Descriptive Statistics (IL-6 pg/ml)			p value
	mean ± SD	std err	min – max	
GDM, BMI <25	2.48 ± 0.8	0.169	2 – 4.74	t =2.49 p=0.016 sig
Control group BMI < 25	2.04 ± 0.1	0.023	2 – 2.37	

t (Student t-test)

5.5 Interleukin 6 in normoglycemic women: BMI > 25 vs BMI < 25

Interleukin 6 had insignificantly higher values in overweight pregnant women from the control group compared to normal weight pregnant women (2.28 ± 0.7 pg/ml vs 2.04 ± 0.1 pg/ml;

p=0.11, table 5).

Table 5: IL-6 in control group and BMI > 25 vs control group and BMI < 25

Control group				
Groups	**Descriptive Statistics (IL-6 pg/ml)**			**p value**
	mean ± SD	**std err**	**min – max**	
Overweight	2.28 ± 0.7	0.1	2 – 5.24	t =1.6
Normal weight	2.04 ± 0.1	0.02	2 – 2.37	p=0.11 ns

Z (Mann-Whitney U Test)

6. Discussion

A large number of studies confirmed that elevated IL-6 can confirm the role of low-grade inflammation in the patogenesis of this disease.

Morriset [5] *et al* measured IL-6 with ELISA method during GDM screenjng and showed significantly higher values of IL-6 in GDM compared to control group (1.47 ± 0.72 vs. 0.90 ± 0.32 pg/mL). Our study showed medium values of this inflammatory parameter 2.77 ± 1.1 pg/ml in GDM vs 2.16 ± 0.5 pg/ml in the control group which was statisticaly significant for p=0.0016.

Metarnal obesity has a growing prevalence worldwide and represents a major obstetric problem increasing maternal and neonatal morbidity and mortality. Wishing to establish the influence of body mass index on this biochemical parameter we made a comparison of pregnant women with GDM and normoglycemic women with BMI more or less than 25. In our study values of interleukin 6 significantly depended on the value of BMI.

Overweight women with GDM had significantly higher medium values of interleukin 6 than GDM women with normal weight, (2.58 pg/ml vs 2.19 pg/ml, p=0.0019).

We made the same comparison in normoglycemic women so established that body mass index did not have significant impact on values of IL-6. IL-6 was insignificantly higher in overweight normoglycemic women compared to normal weight women (2.28 ± 0.7 pg/ml vs 2.04 ± 0.1 pg/ml; p=0.11).

When we compared overweight women, those with GDM had significantly higher interleukin 6 than overweight women without GDM (3.06 ± 1.4 pg/ml vs 2.28 ± 0.7 pg/ml, p=0.021).

Pantam *et al* [13] showed that elevated circulating levels of interleukin 6 in the maternal serum occur in GDM with or without obesity. We confirmed that average value of IL-6 in GDM women with normal weight was 2.48 ± 0.8 pg/ml and significantly higher than normoglycemic women with normal weight, 2.04 ± 0.1 pg/ml, p=0.016 which confirms that even without obesity in GDM there are elevated IL-6 levels.

7. Conclusion

Interleukin 6 in our study had statisticaly significantly higher values in GDM compared to normoglycemic women. Our results showed that both overweight and normal weight pregnant women with gestational diabetes melitus had significantly higher IL-6 than normoglycemic women with same body mass index. However further studies are needed for evaluation of IL-6 concentration throught pregnancy and correlation of this marker to other clinical and biochemical parametars.

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