Immune Inflammation Related to Obesity in Pregnant Women

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Although there are many conditions to be met for the disease to occur (genetic predisposition, environmental factors, stress, exposure to pollutants, noxes, xenobiotics, diet), chronic inflammation is the way how the body responds to these substances. Excess weight leads to an alteration of the immune response, to an increased oxidative stress, and damage to the cellular Deoxyribonucleic acid (DNA) structure. The study aims to evaluate inflammation in obese mothers. The study group consisted of 30 pregnant women in which inflammation was analysed, with a mean age of 30.80 ± 6,94 years old divided in 2 groups depending on their weight, that is 25 pregnant women with obesity and 5 normoponderal pregnant women. From the markers found in the blood samples, only CRP (AUC=0.740; IC95%: 0.466-1.014), leptin (AUC=0.616; IC95%: 0.296-0.936) and glycaemia (AUC=0.648; IC95%: 0.369-0.927) were good indicators of immune inflammation. The estimated risk of immune inflammation is 5 times higher in obese pregnancies with CRP levels above 6.

Keywords: obesity, immune inflammation, pregnant woman.

Excessive fat accumulation in the abdomen is associated with increased cardio-metabolic risk, regardless of age. The mechanisms underlying this process are numerous and have as an essential cause that perivisceral adiposity is metabolically active. Primary and secondary prophylaxis of central obesity through lifestyle changes have a very important role in avoiding non-communicable diseases and its assessment by simple somatic measurements should be part of a prophylactic medical examination [1].

Numerous studies have focused on the causal link between the presence of visceral adiposity and the increase in insulin resistance. However, there is no consensus on how this happens. One possibility may be that visceral adiposity is diabetogenic per se, secreting adipokines that decrease the insulin sensitivity of different tissues, but especially of the liver and muscles, the action being increased as the adipose tissue accumulates. Another possibility is that visceral fat would be an indicator of fat ectopic accumulation and lipotoxicity that occurs in parallel in the liver and muscle, resulting in insulin resistance in these tissues. A third possibility would be that excess lipid accumulation in visceral adipose tissue determines its acquisition of diabetogenic properties. It is proven that visceral fat accumulates macrophages that secrete proinflammatory cytokines that decrease insulin sensitivity. A fourth possibility would be that peripheral tissue lipotoxicity and cytokine secretion of perivisceral fat contribute together to systemic resistance to insulin [2].

The development of obesity is associated with substantial modulation of adipose tissue structure, involving adipogenesis, angiogenesis and remodelling of the extracellular matrix. These processes require proteolytic activity, mainly provided by fibrinolytic (plasminogen / plasmin) systems, matrix metalloproteinase and ADAM (a-disintergin and metalloproteinase) / ADAMTS (a-disintergin and metalloproteinase with thrombospondin motifs). In early development of the adipose tissue, adipogenesis is closely associated with angiogenesis. Thus, the adipose tissue triggers the formation of blood vessels and, in turn, endothelial cells of adipose tissue promote the differentiation of pre-adipocytes. Modulation of angiogenesis and proteolytic systems may have the potential to affect the development of adipose tissue [3]. Associated medical issues developed by the mother are likely to have impact later on the baby too [4-6].

Obesity in pregnancy increases the risk of certain complications such as [7-13]:

Gestational diabetes. Obese women are at increased risk of having diabetes than women with normal weight.

- Preeclampsia. Obesity increases the risk of developing high blood pressure and excess protein in the urine after the 20th week of pregnancy.

- Infections. The risk of urinary tract infections is increased. In addition, obesity increases the risk of postpartum infections, whether the baby is born vaginally or by caesarean.

- Thrombosis. Women who are obese during pregnancy have an increased risk of developing thrombi (blood clots) in the deep veins of the lower limbs, a serious condition that may be complicated by pulmonary thromboembolism.

- Obstructive sleep apnoea syndrome. It is a condition in which, during sleep, breathing stops for long periods of time.

- Prolonged pregnancy. Obesity increases the risk that pregnancy may extend beyond the 42-week term.

- Problems during labour. In obese pregnant women the induction of labour is much more common. Obesity may interfere with the use of certain pain control methods such as epidural anaesthesia.

- Caesarean section. Obesity increases the risk of elective caesarean surgery or in an emergency. Also, complications of caesarean surgery are more common, such as delayed wound healing or infections at incision level.

- Loss of pregnancy. Obesity has an increased risk of abortion or the birth of a dead child.

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Purpose and objectives

Obesity in pregnancy causes serious health problems for the child:

- Macrosomia. Obese women have a significant risk of having children that are larger than the average (macrosomes) and have a higher body fat than normal. Research has also shown an increased risk of obesity in childhood.

- Chronic conditions. Obesity in pregnancy increases the risk of the baby suffering from heart disease or diabetes as an adult.

- Congenital defects. There is a slightly increased risk of congenital heart disease or neural tube defects.

One of the main objectives of the study was to identify correlation between leptin levels during pregnancy in obese women with inflammatory markers. Changing the lifestyle can contribute to a healthier metabolic profile in obese pregnant women, which will reduce the risk of serious health problems in the product of conception.

Experimental part

Material and method

The study was a prospective, case control one, performed on a group of 30 pregnant women who gave birth to babies in the Obstetrics and Gynaecology Clinic belonging to Elena Doamna Hospital Iasi and were assessed the degree of immune inflammation associated to obesity.

The group studied consisted of 30 pregnant women, obese (n=25), overweight and normal weighted (n=5), who were harvested blood samples to determine the level of markers associated with the inflammatory process: *C-reactive protein* (CRP), leptin, insulin, glucose, Homeostatic Model Assessment for Insulin Resistance (HOMA).

C-reactive protein is a plasmatic protein of the pentraxin family and an acute phase reactant, very useful as a general inflammation marker. CRP levels above the cut point generally used to indicate an obvious source of infection or inflammation >6 mg/dL.

Patients were sampled by venous puncture for determination:

leptin, determined by the immunoenzymatic method, Elisa Sunrise Tecan equipment, reference values 4-25 ng/mL;

insulin, determined by the Chemiluminiscent method, Siemens Immulite 2000 XPI equipment, reference values 2.6-24.9 µU/mL;

glugose, determined by the Spectrophotometric method, Abbott Architect c8000 equipment, reference values 80-120 mg/dL.

HOMA-IR Blood Code Calculation = Insulin (µU/mL) x Glucose (mg/dL).

The data were uploaded and processed using statistical functions in SPSS 18.0 at the significance threshold of 95%.

Significance tests. Skewness or Kurtosis tests (-2 < p < 2) are those tests measuring the normalcy of the set of values in order to establish if the variables are continuous or not.

In calculating the significant difference between two or more groups, based on the distribution of the series of values, for the significance threshold of 95% and for the quantitative variables, we applied the t-Student test - a parametric test that compares the average values recorded in 2 groups with normal distributions.

The χ^2 test is a nonparametric test comparing 2 or more frequencies distributed from the same population and applies when the expected events are excluded. Sometimes, when the frequency calculation formula is small, Yates correction formula is applied to obtain a higher estimate of the difference. The Kruskall-Wallis correlation compares ordinal variables from 3 or more groups.

Pearson correlation coefficient (r) represents the correlation of 2 variables in the same group, the direct / indirect correlation being given by the coefficient sign.

The *Receiver Operating Characteristic (ROC)* curve highlights the predictability of some laboratory markers in the determinism of immune inflammation in pregnant women.

Results and discussions

Epidemiological characteristics

Weight status

Body mass index (BMI) varied from 18.79 to 44.14 kg/m^2, with a coefficient of variance of 29.16%, the mean value of the group being 34.17 ± 5.40 kg/m^2, close to the median value (33.90 kg/m^2). The results of Skewness (p= -0.403) and Kurtosis tests (p=1.014) suggest the normal distribution of the set of BMI values recorded (fig. 1).

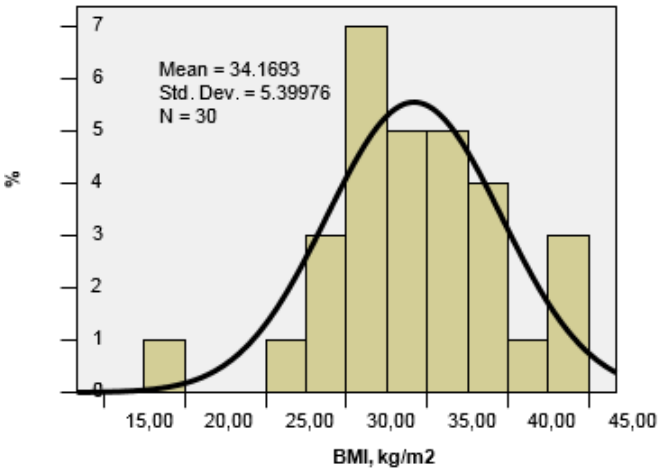

Fig. 1. BMI Histogram

The average level of the BMI set of values close to the obesity threshold (34.9 kg/m^2) provided by the specialized literature leads to the group being split in two:

Group I - 25 women with the level of BMI over 34.9 kg/m^2, the mean value of the lot being 35.73±4.12 kg/m^2

Group II - 5 women with the level of BMI below 34.9 kg/m^2, the mean value of the lot being 26.39±4.35 kg/m^2

Distribution by age group revealed that 56% of the total obese patients (group I) were in the 30-39 age group, while 60% of the total group II was in the 20-29 age group (Chi-square=1.48; df=2; p=0.477).

Maternal pathology

Based on the cases studied, the maternal pathology associated with obesity did not induce an estimated higher risk (table 1), but the issue could be reconsidered on a larger number of patients enrolled in a future study.

Gestational hypertension (HTA)- pregnancy-induced hypertension - was noted in 20% of obese patients and 20% of non-obese patients (p=1.000).

The scarring uterus was more commonly encountered in obese patients (48% vs. 40%, p = 0.743), but the percentage distribution did not show statistically significant differences.

Diabetes and placenta previa were noted in only 2 obese patients. The reduced number of cases does not allow extrapolation of the results to the general population.

Stopped birth mechanism was more frequently associated with non-obese patients (16% vs 40%; p=0.252).

Table 1
THE RISK OF OBESITY CORRELATED WITH MATERNAL PATHOLOGY

Maternal pathology	Group I (n=25)		Group II (n=5)		Chi2	p	OR	RR	IC95%
	n	%	n	%					
Gestational HTA	5	20.0	1	20.0	0.000	1.000	1.00	1.00	0.67-1.49
Diabetes	2	8.0	0	0.0	0.757	0.384	-	1.22	1.02-1.45
Scarred uterus	12	48.0	2	40.0	0.108	0.743	1.39	1.06	0.77-1.45
Placenta previa	2	8.0	0	0.0	0.757	0.384	-	1.22	1.02-1.45
Stopped birth mechanism	4	16.0	2	40.0	1.311	0.252	0.27	0.76	0.42-1.37

In conclusion, presence of gestational hypertension, scarring uterus, diabetes and placenta previa the maternal pathology were not risk factors for the pregnant woman.

Factors related to foetal development (table 2)

Gestational (pregnancy) age varied from 34 to 41 weeks, with a variation coefficient of 4.1%, the mean value of the group being 37.47 ± 1.55 weeks, with a slightly reduced mean for the obese patients (37.44 vs 37.60 weeks; p=0.837).

The weight of the foetus ranged from 2700 to 4290 g, with a variation coefficient of 11.7%, the mean value of the group being 3440 ± 403 g, with a slightly increased mean for the obese patients (3461 vs 3336 g; p=0.537).

The Apgar Score varied from 6 to 9, with a variation coefficient of 10.1%, the mean value of the group being 8.53 ± 0.83, without significant differences depending on the mother's weight status (8.44 vs 9.0; p=0.189).

Lab markers

C-reactive protein (CRP)

From the obese patients only 12% showed a level of CRP<6. The Chi2 test revealed that the estimated risk (RR) of immune inflammation in obese patients with CRP>6 was over 5 times bigger (RR=5.1; IC95%: 1.38-17.39; p=0.026) (fig. 2).

Leptin varied from 0.62 to 61.84 ng/mL, with an ample variance coefficient (101.81%), the mean value of the group being of 15.45 ± 15.73 ng/mL, much bigger than the median value (9.24 ng/mL), which suggests the fact that

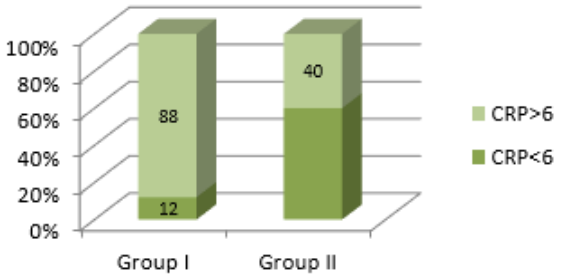

Fig. 2. The structure of the groups by level of CRP

the set of values did not have a normal distribution, aspect also confirmed by the results of the Kurtosis test (p=2.167) (table 3).

Insulin varied from 0.51 to 25.42 µU/mL, with an ample coefficient of variance (108.4%), the mean value of the group being 5.11 ± 5.54 µU/mL, much bigger than the median value (3.35 ng/mL), which suggests the fact that the set of values did not have a normal distribution, this aspect being also confirmed by the results of the Skewness tests (p=2.562) and Kurtosis tests (p=6.700) (table 3).

Blood glucose varied from 66 to 116 mg/dL, with a coefficient of variance of 14%, the mean value of the group being of 85.01 ± 11.90 mg/dL, close to the median value (83.40 mg/dL), which suggests the fact that the set of values had a normal distribution, this aspect being also confirmed by the results of the Skewness tests (p=0.749) and Kurtosis tests (p=0.165) (table 3).

Homeostatic Model Assessment for Insulin Resistance (HOMA) varied from 0s11 to 6.57, with a coefficient of

Characteristics	Group I	Group II	t-Student test (p)
BMI, kg/m²	35.73±4.12	26.39±4.35	0.001
Age, y	31.04±7.09	29.60±6.69	0.679
Gestational age, weeks	37.44±1.64	37.60±1.14	0.837
Weight of the foetus, g	3461±407	3336±413	0.537
Apgar score	8.44±0.92	9.00±0.01	0.189

Table 2
COMPARATIVE EPIDEMIOLOGICAL CHARACTERISTICS OF STUDIED GROUPS

Statistical indicators	Leptin (ng/mL)	Insulin (µU/mL)	Glucose (mg/dL)	HOMA
N	30	30	30	30
Mean	15.45	5.11	85.01	1.12
Median	9.24	3.35	83.40	0.72
Std. deviation	15.73	5.54	11.90	1.37
Variance	101.81	30.67	141.61	1.88
Skewness	1.569	2.562	0.749	2.905
Std. Error of Skewness	0.427	0.427	0.427	0.427
Kurtosis	2.167	6.700	0.165	8.932
Std. Error of Kurtosis	0.833	0.833	0.833	0.833
Range	61.24	24.91	50	6.46
Minimum	0.61	0.51	66	0.11
Maximum	61.84	25.42	116	6.57
Percentiles 25	3.32	2.13	75.55	0.40
50	9.24	3.35	83.40	0.72
75	22.53	4.96	91.28	1.00

Table 3
STATISTICAL INDICATORS OF THE LAB MARKERS

variance of 122.3%, the mean value of the group being of 1.12 ± 1.37, different from the median value (0.72), which suggests the fact that the set of values did not have a normal distribution, this aspect being also confirmed by the results of the Skewness (p=2.905) and Kurtosis tests (p=8.932) (tab.3).

The individual level of leptin correlated directly with BMI, showing the fact that in 21.8% of pregnancies the higher values of leptin were met when we recorded increased values of the BMI (r= +0.218; R^2=0.0473; p= 0.248), but the result cannot be extrapolated to the general population. The level of insulin and the BMI were apparently independent parameters (r= +0.042; R^2=0.0018; p= 0.825). The individual level of glycaemia correlated directly with the BMI, which was reduced as intensity, showing the fact that in 10,6% of the pregnancies the increased values of glycaemia were associated with increased values of the BMI (r= +0.106; R^2=0.0107; p= 0.576) (fig. 3).

HOMA Index and BMI were apparently independent parameters (r= +0.091; R^2=0.0083; p= 0.632).

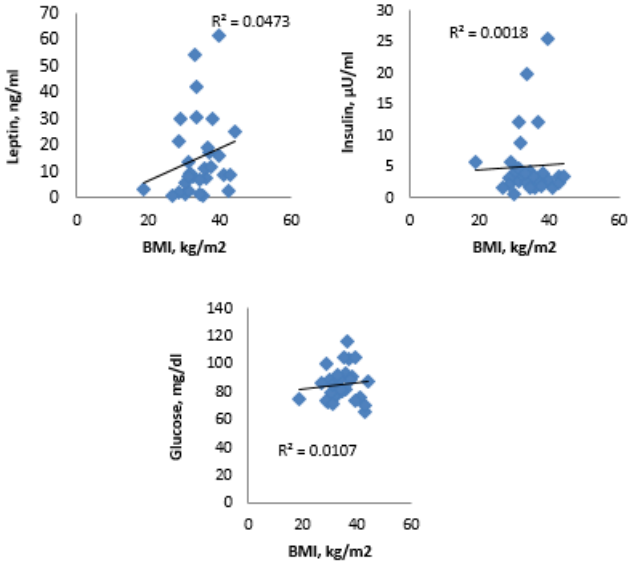

Fig. 3. The correlation of leptin, insulin and glycaemia with BMI

Assessing eating habits. The Disabilities of the Arm, Shoulder and Hand (DASH) Score

DASH score varied from 14 to 27, with a variation coefficient of 15.1%, the mean value of the group being 22.07 ± 3.33, which is close to the median value (22.50), suggesting the fact that the set of values had a normal distribution. This aspect is also confirmed by the results of the Skewness (p= -0.584) Kurtosis tests (p= -0.114) (fig 4).

DASH score correlated indirectly with the BMI, showing the fact that 30.6% of the pregnant women associate more reduced values of the DASH score with increased values of the BMI (r= -0.306; R^2=0.0934; p= 0.05), (fig. 5).

For the group of obese patients, the mean level of DASH score was significantly reduced (21.68 vs 24.0; p=0.05) (table 4).

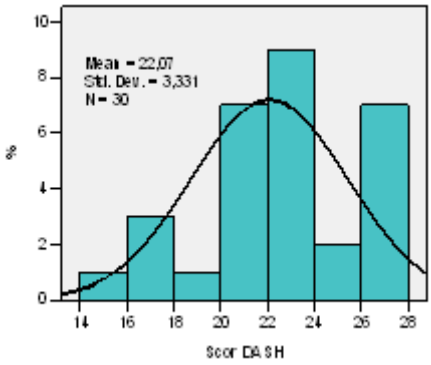

Fig 4. Histogram of DASH score

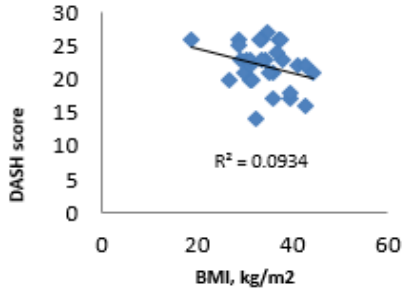

Fig 5. The correlation of DASH score with BMI

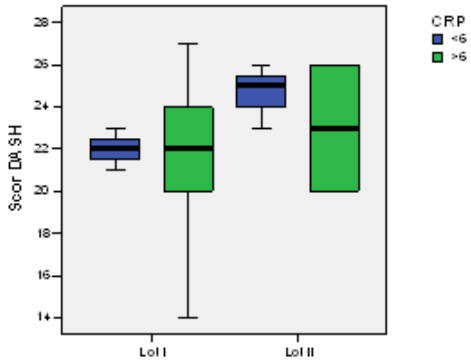

Fig. 6. Correlation of the mean DASH score with the CRP level compared to study lots

The mean DASH score was not significantly different depending on the CRP level (p>0.05) (fig. 6).

The DASH score correlated directly, but reduced in intensity, with the level of leptin (r= +0.165; R^2=0.0272; p= 0.384), but the result cannot be extrapolated to the general population. The insulin level and the DASH score were apparently independent parameters (r= -0.082; R^2=0.0067; p= 0.666). The individual level of glycaemia correlated directly with the DASH score, which was reduced as intensity, showing the fact that 22.3% of the pregnant women had increased values of glycaemia associated with increased values of the DASH score (r=+0.223; R^2=0.0501; p= 0.235) (fig. 7).

Table 4

DESCRIPTIVE INDICATORS OF DASH SCORE COMPARED ON GROUPS STUDIED

Group	N	Mean	Std. Deviation	Std. Error	Confidence interval 95%		Min	Max	p - Test t-Student
					-95%CI	+95%CI			
Group I	25	21.68	3.375	0.675	20.29	23.07	14	27	0.050
Group II	5	24.00	2.550	1.140	20.83	27.17	20	26	
Total	30	22.07	3.331	0.608	20.82	23.31	14	27	

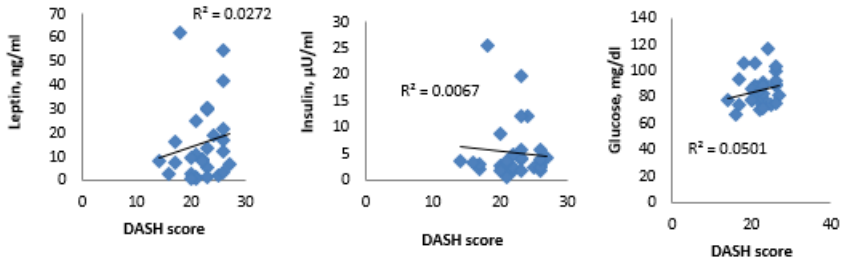

Fig. 7. Correlation of the mean DASH score with the CRP level compared to study lots

Table 5

THE ROC CURVE COORDINATES FOR OBESE PREGNANT WOMEN

Test Result Variable(s)	Area under curve (AUC)	Std. Error(a)	Asymptotic Sig.(b)	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
CRP	0.740	0.140	0.095	0.466	1.014
Leptin, ng/ml	0.616	0.163	0.420	0.296	0.936
Insulin, µU/ml	0.536	0.149	0.802	0.244	0.828
Glycaemia, mg/dl	0.648	0.142	0.303	0.369	0.927
HOMA	0.496	0.142	0.978	0.217	0.775

HOMA level and DASH score were apparently independent parameters (r= -0.079; R^2=0.0063; p= 0.676).

By drawing the ROC curve on the cases studied, we noticed the fact that of the markers determined from the blood samples only CRP (AUC=0.740; IC95%: 0.466-1.014), leptin (AUC=0.616; IC95%: 0.296-0.936) and glycaemia (AUC=0.648; IC95%: 0.369-0.927) were good indicators of immune inflammation (table 6, fig. 8).

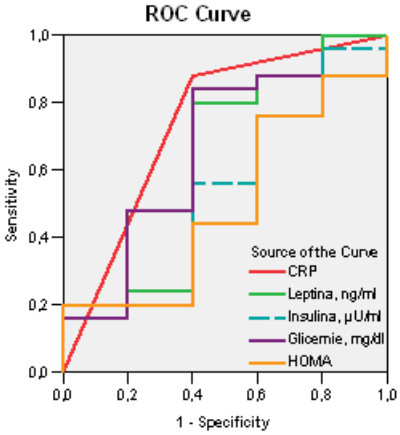

Fig. 8. The balance sensitivity / specificity of Laboratory Parameters in identifying the immune inflammation in obese pregnant women

-CRP: cut off value 6, sensitivity 88%, specificity 62%

-Leptin: cut off value 6.27 ng/mL, sensitivity 79%, specificity 60%

-glucose: cut off value 80 mg/gL, sensitivity 82%, specificity 60%.

The test result variable(s): CRP has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

a Under the nonparametric assumption

b Null hypothesis: true area = 0.5

Beyond the mechanisms, however, it is clear that the risks of abdominal obesity (quantified by abdominal circumference and waist / hip ratio) are significant. Abdominal obesity is an important factor associated with immune inflammation and various metabolic disorders, independent of other factors, including body mass index [2, 12].

During pregnancy in obese women we have identified significant changes in leptins and more modest changes in insulin or glucose with some evidence that this is more pronounced than in pregnant women with a normal weight [13].

Autophagy in adipose tissue of obese people correlates with obesity, visceral fat distribution and adipocyte hypertrophy. This may coincide with insulin resistance but precedes the occurrence of obesity related morbidity [11].

Conclusions

The group that was studied was made of 30 pregnant women with immune inflammation, with a mean age of 30.80 ± 6.94 years old.

The maternal pathology of the obese pregnant group was dominated by the scarred uterus (48%) and pregnancy induced blood hypertension (20%). We have also identified some cases where the birth mechanism stopped (16%), placenta previa (8%) and associated diabetes (8%).

In the group of obese pregnant women, factors related to the development of the foetus revealed an average gestational age of 37.44±1.64 weeks, a mean weight of the foetus of 3461±407 g and the mean Apgar score of 8.44±0.92.00

The estimated risk of immune inflammation is 5 times higher in obese pregnant women with a CRP value over 6.

The DASH score varied from 14 to 27, with a mean value of 2.07 ± 3.33, significantly reduced in obese patients (21.68 vs 24; p=0.05).

About 30% of the pregnant women associated higher values of BMI with higher values of leptin and glycemia.

The change in the lifestyle can contribute to a healthier metabolic profile for the obese pregnant women, but the results require further studies.

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