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Research Article

Association of serum nitric oxide, free beta human chorionic gonadotropin and body mass index in first trimester pregnancy

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ABSTRACT

Background: Pregnancy is a state of oxidative stress which arises from increased placental mitochondrial activity and production of reactive nitrogen species mainly nitric oxide (NO). NO is a potent vasodilator, which have pronounced effects on placental function which includes proliferation and differentiation of trophoblast. Human chorionic gonadotropin (hCG) is secreted by the syncytiotrophoblas. During pregnancy, free βhCG level can be first detected in maternal blood from 11th day after conception. Increased in NO synthesis acts as endocrine modulator of the placenta which promote secretion of free β hCG. The objective of this study was to estimate and compare the levels of βhCG and NO with different groups of BMI of pregnant women in first trimester.

Methods: The study group comprises of 85 pregnant women within the age of 20-40 years with singleton pregnancy (11th-13th week + 6 days of gestation) who came for routine first trimester screening. Serum levels of free β hCG was analyzed by electrochemiluminiscense. NO was measured by kinetic cadmium reduction method. Statistical analysis used: data was expressed as mean±SD and median. Comparisons between different groups of BMI were done using Kruskal Wallis test.

Results: There was significantly increased level serum NO with the increase in BMI and significantly decrease in serum levels of ($p>0.05$) free βhCG. We found significant positive correlation between NO and βhCG ($p>0.05$, r value 0.01).

Conclusions: Early placental formation requires high amounts of angiogenesis and vasculogenesis. Its initiation, maturation, and maintenance are of critical importance. Failure to placental formation can lead to preeclampsia and/or intrauterine growth restriction (IUGR).

Keywords: First trimester pregnancy, Free beta hCG, Nitric oxide, BMI

INTRODUCTION

Initial development of vascular system of placenta is major process for adequate fetal development. Pathologies related to pregnancy such as pre-eclampsia, eclampsia and IUGR are related with vascular dysfunction in the placental formation.[1] Nitric oxide (NO) is biologically active molecules that are synthesized from L arginine by the enzyme nitric oxide synthase (NOS). It is an endogenous molecule that acts as a mediator, a hormone, a reactive oxygen species (ROS).[2,3] Placenta, which is the major source of nitric oxide (NO) production, mediates placental vasculogenesis and angiogenesis which regulate embryo development, implantation, trophoblast invasion and fetal growth throughout gestation.[4-7] A decrease in NO production is caused by lipid peroxidation and nitrosylation of molecules which stimulates oxidative phosphorylation and increase in localoxygen uptake Szabo C et al.[8-10]

The human chorionic gonadotropin (hCG) is a glycoprotein composed of two non-covalently linked

subunits, α and β, and is produced by syncytiotrophoblast cells of the placenta.[11,12] Maternal serum hCG peaks at 8-10 week of gestation and then declines to reach a plateau at 18-20 week of gestation.[13] The free β-subunit can derive from three sources, namely, direct trophoblast cell production, dissociation of hCG into free α- and free β-subunits, and by macrophage or neutrophil enzymes nicking the hCG molecule.[14-16]

The free β-hCG circulating in maternal serum corresponds to only about 0.3-4% of the total hCG.[17,18] During pregnancy, free beta hCG level can be first detected by a blood test at about 11 days after conception.[19] The other studies have shown that hCG functions to promote angiogenesis and vasculogenesis in the uterine vasculature during pregnancy. This ensures maximal blood supply to the invading placenta and optimal nutrition to the fetus.[20-26]

Winfried Rossmanith G et al has investigated the expression and localization of enzyme nitric oxide synthase (eNOS) and the effect of NOS on placental human chorionic Gonadotropin (HCG) release. They found that there was increased production of nitric oxide during gestation period. Thus, NOS is expressed in the human placenta at increasing levels during gestation. Increased in NO synthesis acts as endocrine modulator of the placenta which promotes secretion of HCG.[27] Since NO and free βhCG are of placental origin and NO increases the secretion of free βhCG during first trimester.[27] Therefore, the aim of the present study was to estimate and compare the levels of βhCG and NO with different groups of BMI of pregnant women in first trimester.

METHODS

Subjects in this study were Institutional Ethics Committee permission and consent from participants was obtained before carrying out this study. This study was conducted in the department of biochemistry, Kasturba medical college Manipal, Manipal University, during the period from September 2014 to February 2015. This prospective study includes 85 females, who were enrolled for first trimester screening programme at Kasturba medical college, Manipal, Manipal University. Pregnant women aged 20-40 years in 11-13 weeks + 6 days of gestation who come for first trimester screening were included in the study. Pregnant women with history of gestational diabetes, Hypertension, liver disease and renal failure were excluded from study.

Blood samples were collected into empty red vacutainer and immediately stored on ice at 4°C. The serum was then separated from the cells by centrifugation at 3,000 rpm for 10 minutes. Serumsamples used for the measurement of free βhcGand NO levels and were stored at -80°C until they were used.

Serum free βhcG was measured by electrochemilluminiscense method in COBAS 6000 analyser.[27]

Measurement of serum nitric oxide

Serum nitric oxide by measured by kinetic cadmium reduction test.

Nitric oxide production was determined by the evaluation of its oxidation products (nitrites and nitrates), where nitrates were reduced to nitrites with cadmium fillings, the total concentration of nitrites was then measured by using Griess reaction.[28]

Measurement of Body Mass Index (BMI)

Height and weight of pregnant women was taken and BMI was calculated by following formula.[29]

BMI = Weight in kg/height in m^2

Statistical analysis

The data analysis was done by using SPSS statistic analyser software version 19. Data was expressed as mean±SD and median (inter quartile range, IQR). Comparisons between different stages of cancer were done using Kruskal Wallis test. Pairwise comparisons was done using Mann Whitney test with Boneferroni correction for type I error.

RESULTS

Table 1: Clinical characteristics of cases.

Variables	Mean±SD
Number of pregnant women	85
Maternal age (years)	29.10±3.70
Weight (kg)	53.73±9.71
Height (cm)	155.55±6.20
BMI ($kg/m^{2)}$	22.23±3.90
NO	148.83±26.90
BhCG	32.08 (22.36, 53.74)*

Median (interquartile range)*

Table 2: Comparison of level of serum nitric oxide in different BMI groups.

Group	BMI kg/m^2	Number of cases	Nitric oxide (µmoles/L) (mean ±SD)	*p value
1	21-25 Normal	48	130.70± 3.20	NS
2	26-28 Over weight	17	141.70±31.30	NS
3	29-36 Obese	03	150.90±14.17	0.05

*Mann-whitney test

The clinical parameters, serum β-hCG and NO level of enrolled mothers were tabulated and compared with different BMI groups of pregnant women. p value less than 0.05 was considered as statistically significant.

- P value group 1 versus group 2-Not significant (NS)
- P value group 2 versus group 3-Not significant
- P value group 3 versus group 1-significant
- Boneferroni correction for alpha error is used for multiple pair wise comparisons.

Table 2: Comparison of level of free βhCG in different BMI groups.

Group	BMI kg/m^2	Number of cases	BhCG (ng/L) (median)	*p value
1	21-25 Normal	48	33.15	NS
2	26-28 Over weight	17	28.33	NS
3	29-36 Obese	03	22.00	0.05

*Mann-whitney test

- P value group 1 versus group 2-Not significant (NS)
- P value group 2 versus group 3-Not significant
- P value group 3 versus group 1-Significant
- Bonneferoni correction for alpha error is used for multiple pair wise comparisons.

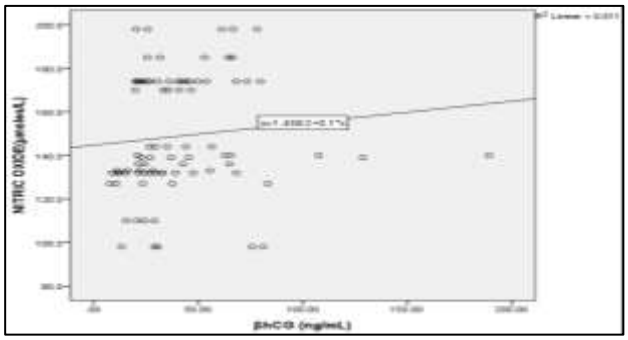

Figure 1: Scatter plot of positive correlation between NO and free βhCG (r value 0.011).

DISCUSSION

In the present study serum levels of free βhCG and NO were compared between different BMI groups of pregnant women. Our study showed the mean level of serum NO was significantly increased as the advancement of BMI. This was in accordance with Fujita et al and Ghasemi A et al who found an increase level of serum NO with increased BMI. They had explained that enzyme NOS is present in human adipose tissue which produce NO, which in turn regulates energy balance and food intake by regulating lipolysis in human body.[30] Further our study showed that there was significantly decrease in serum levels of (p>0.05) free βhCG. The lower free βhCG serum concentrations in obese compared with lean women was due to fact that obese women have a more extracellular volume, and hence they have high capacity for βhCG in extracellular space. Furthermore, Fiete D et al had demonstrated that the low hCG in women with high BMI may be sequestration of hCG by adipose tissue resident macrophages.[31] Since NO and βhCG are of placental origin and are involved in vasculogenesis and angiogenesis to supply optimum blood flow to the placenta. Hence we also tried to correlate the levels of these two parameter in pregnancy. We found significant positive correlation between NO and βhCG (p>0.05, r value 0.01).

The limitations of this study were the findings of our study cannot be widely accepted due to small sample size. Serum NO may alter in any diseased or inflammatory condition. Further if birth weight of the baby would have considered here it might have given us valid information.

CONCLUSION

Early placental formation requires high amounts of angiogenesis and vasculogenesis. Its initiation, maturation, and maintenance are of critical importance. Failure to placental formation can lead to preeclampsia and/or intrauterine growth restriction (IUGR). Normal placental function is critical for normal fetal development. Understanding the mechanisms which regulate vasculogenesis and angiogenesis in placenta is therefore of critical importance. So, we conclude that measurements of serum levels of NO in the 1st trimester pregnant women will be useful for predicting various late pregnancy complications that are at increased risk of preeclampsia, IUGR and GDM

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