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Original Article

Hyperglycocylated Human Chorionic Gonadotropin Percent to Beta HCG in Preeclampsia in Late Pregnancy

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Abstract:

Background: *Preeclampsia is a multi-systemic disease with incomplete understanding of etiology till now. Detection of preeclampsia is essential in prevention of co-morbidities and death for both mother and fetus. Human chorionic gonadotropin is important in development of placenta.*

Aim of study: *To assess the percent level of hyperglycosylated human chorionic gonadotropin among pregnant women with preeclampsia.*

Patients and methods: *This study is a descriptive case control study carried out in Obstetric department of Baghdad Teaching Hospital in Baghdad-Iraq. The duration of study was six months throughout the period from 1st of July, till 31st of December, 2020 on convenient sample of 100 pregnant women (50 pregnant women with preeclampsia and 50 healthy pregnant women as control). The women were assessed by the senior and the researcher. The diagnosis of preeclampsia was confirmed according to American College of Obstetricians and Gynecologists guidelines by measuring blood pressure of 140/90 mmHg and over in two occasions 4 hours apart after 20th weeks of gestation with previous normal blood pressure.*

Results: *Mean beta human chorionic gonadotropin of pregnant women with PE was significantly higher than beta human chorionic gonadotropin of healthy controls (p=0.002). Mean hyperglycosylated human chorionic gonadotropin of pregnant women with PE was significantly higher than mean hyperglycosylated human chorionic gonadotropin percent of healthy controls (p<0.001). Mean hyperglycosylated human chorionic gonadotropin percent of pregnant women with PE was significantly higher than mean hyperglycosylated human chorionic gonadotropin percent of healthy controls (p=0.03). Cutoff point of hyperglycosylated human chorionic gonadotropin ratio of 1.9 had acceptable validity results.*

Conclusions: *The hyperglycosylated human chorionic gonadotropin percent were increased significantly in preeclampsia at late pregnancy.*

Keywords*: Preeclampsia, Hyperglycosylated Human Chorionic Gonadotropin percent, Late pregnancy.*

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1. INTRODUCTION

Preeclampsia is a multisystem syndrome of variable severity, specific to the pregnancy characterized by a reduction in the systemic perfusion generated by vasospasm and activation of coagulation systems. It is presented after the 20th week of gestation, during childbirth or in the two weeks after this one (1). Preeclampsia is a pathology with a worldwide prevalence of 10% of pregnancies. Presents a major incidence in women under 20 years and over 35 years old at the moment of the pregnancy and of these 75% of the cases correspond to patients primal. It was previously classified as mild, moderate and severe pre-eclamsia but within the new classification is only talked about preeclampsia with criteria of severity or no criteria of severity. It is a pathology that in most cases she is asymptomatic and when she presents symptoms usually have criteria of severity so it is important the control of blood pressure within the prenatal checks (2,3). Many mechanisms have been described pathological for the appearance of the preeclampsia but even the etiology does not it's totally clear. The main mechanism described that the explains is the trophoblastic invasion abnormal of the uterine vessels. As well immune intolerance has been described between maternal and fetoplacental tissues, poor adaptation of the mother to changes inflammatory and cardiovascular of the normal pregnancy and genetic influences (4). There is a defect in the placentation and a failure to reorganize the arteries spirals which leads to the release of circulating toxic factors with endothelial dysfunction, vasoconstriction and state of hypercoagulability. As a result of this procedure high blood pressure occurs, glomerular injury with proteinuria, thrombocytopenia, hemolysis, ischemia hepatic, ischemia of the nervous system central and finally eclampsia with seizures (5,6). The human chorionic gonadotropin hormone, choriogonadotropin or human chorionic gonadotropin (hCG) is a protein synthesized mainly by embryonic tissues; It is made up of 2 amino acid chains called alpha (α) and beta (β), non-covalently linked by a sulfhydryl bridge, which, if separated, lose their biological activity; that is to say, none of them have activity by themselves, but they recover it when they recombine (7). The α subunit is common to other hormones such as luteinizing hormone (LH), follicle-stimulating hormone (FSH), pituitary thyrotropin (TSH); while β is different from any other hormone and is the one that gives it its specificity. Its molecular weight had been calculated between 36,000 to 40,000 (8). Its secretion is related to the mass of trophoblastic tissue, being correlated with trophoblastic extension from 4 to 20 weeks and with weight from 20 to 28 weeks, in such a way that the

rapid elevation between 3-9 weeks of pregnancy coincides with the proliferation of immature trophoblastic villi and an extensive syncytial layer (9). The decrease in the amount of trophoblastic tissue typically seen between 10-18 weeks is also associated with a decrease in serum hCG concentration. From there, at the end of pregnancy, there is an increase in the hCG dimer that is proportional to the size of the placenta and chorionic villi; this means that its elevation is due to proliferation and placental invasion. Serum and urine hCG concentrations vary substantially during pregnancy and between individuals; it is of little use for "dating" a pregnancy from the last day of the menstrual period. Its variations are considered to be the largest of any other hormone or its metabolites in healthy individuals. Concentrations as low as 20 mIU/mL or as high as 8,900 mIU/mL have been reported in the fifth week of pregnancy, leading to normal term deliveries (10). In urine the variations have been reported even larger, concentrations in the 5th. week from 22.8 mIU/mL to 41.95 mIU/ml. Among the explanations for these wide variations is the one that establishes that when a small amount of cellular receptor is activated, a response similar to when all are activated may occur, causing limitations in cellular adenosine monophosphate, protein kinase, or G-protein responses; theory known as the "LH/hCG receptor sparing or receptor sparing phenomenon". Hyperglycosylated human chorionic gonadotrophin is a variant type of hCG glycosylation that formed by root cytotrophoblast cells and extravillous cytotrophoblast cells (11).

2. PATIENTS AND METHODS

Study design and settings

This study is a descriptive case control study carried out in Obstetrics department of Baghdad Teaching Hospital in Baghdad-Iraq. The duration of study was six months throughout the period from 1st of July, till 31st of December, 2020. All pregnant women with symptoms and signs of preeclampsia admitted to Gynecology department of Baghdad Teaching Hospital were the study population.

The Inclusion Criteria:

- Adult women (18-39 years)
- Singleton pregnancy
- Gestational age (20-40 weeks) with preeclampsia.

The Exclusion Criteria:

• Age >40 years

• Multiple pregnancy

• On treatment of preeclampsia

• Other chronic diseases, malignancy, renal failure, liver diseases, hematological diseases, autoimmune diseases, endometriosis and refuse to participate in the study.

After eligibility to inclusion and exclusion criteria, a convenient sample of 50 pregnant women with preeclampsia was selected from Consultancy Clinic after taking their approval to participate in the study. A sample of 50 adult healthy pregnant women with gestational age (32-40 weeks) was selected from Consultancy Clinic of Baghdad Teaching Hospital.

Data Collection:

All respondents variables were collected by using a specially designed questionnaires and through face to face interview

The questionnaire included the followings:

1. Age of participants.
2. Gestational age at presentation.
3. Physical examination: It included the examination of followings vital signs: Blood pressure of pregnant women that was measured by sphygmomanometer with a cuff of covered size:
    a) The women should seat in comfortable position.
    b) The cuff should be putted with lower end of arm above the antecubital fossa by 2.5 cm.
    c) 20-30 mmHg inflation of the cuff above the palpable systolic blood pressure.
    d) Deflation of cuff in rate of 2 mmHg/second.
    e) Systolic blood pressure was recorded via auscultation.
    f) Diastolic blood pressure was recorded via Krotkoff sound.
4. Proteinuria: Albumin in urine (No, +, ++ or +++).
5. Serum uric acid.
6. Serum creatinine.
7. Blood urea.
8. Serum beta human chorionic gonadotropin and hyperglycosylated human chorionic gonadotropin (hCG-H).

The women were assessed by the researcher. The diagnosis of PE was confirmed according to ACOG guidelines by measuring blood pressure of 140/90 mmHg and over in two occasions 4 hours apart after 20th weeks of gestation with previous normal blood pressure (13).

Preparation and Transfer of the Sample

A five cc blood sample was drawn from each enrolled women by the nurse and collected in EDTA tubes then centrifuged in laboratory of the hospital and the samples were frozen into –40C° within 48 hours from collection and saved in the refrigerator of the laboratory, then after collection of all samples they transferred by special portable cool box (Nomad Extreme Cooler) into private laboratory for analysis by ELISA test (Biotek ELx800).

hCG-H kit

The kit used is ELISA Kit (B-hCG). The B-hCG ELISA kit applies the competitive enzyme immunoassay technique utilizing a polyclonal anti-hCG antibody and an hCG-HRP conjugate. The assay sample and buffer are incubated together with hCG-HRP conjugate in pre-coated plate for one hour. After the incubation period, the wells are decanted and washed five times. The wells are then incubated with a substrate for HRP enzyme. The product of the enzyme-substrate reaction forms a blue colored complex. Finally, a stop solution is added to stop the reaction, which will then turn the solution yellow.

The hCG-H units were in pico-milligram/ml that transferred into mIU/ml by online Gem4me electronic program (ENDMEMO) to become applicable in developing of hCG-H/beta HCG ratio required in analysis.

Ethical considerations:

- An approval was taken from Genecology and Obstetrics Scientific Council of Board.
- An oral informed consent was taken from women.
- Management of women accordingly.

Statistical analysis

Data were analyzed after filling of the Microsoft excel program and then change to Statistical Package for Social Sciences (SPSS) version 25. The results arranged in scales variables (means & SD) and in categorical variables. Independent sample t-test was used to compare between two means. Receiver operator curve was used to expect the finest cutoff values of hCG-H in diagnosis of PE. P value of 0.05 or less was regarded as significant.

3. RESULTS

A total of 50 pregnant women with preeclampsia (PE) were included in the study with mean age of 23.1 ± 4.9 years, in addition to 50 apparently healthy women as control group with mean age of 22.6 ± 45.1 years. Both groups were almost matched for maternal and gestational age , (P. value > 0.05), (**Table 1).**

Comparison of blood pressure and albumin in urine of PE group vs. controls, revealed a higly significant higher blood pressure and albumin in urin of PE group compared to controls, where only two PE women had normal blood pressure compared to 47/50 controls, mild hypertension reported in 44% PE women and 3 controls only. Moderate to severe hypertension reported only in PE group; 52% compared to none of controls, in all comparisons, P. value < 0.001. Similarly for albumin in urin where vast majority of controls had no albumin in urine and only 5 controls had one plus. In PE group, the opposite is correct, where only 8 women had no albumin in urine, one plus in 20%, 2 pluses in 56% and 3 pluses in 8%, P. value < 0.001), (**Table 2**). Comparison of mean values of laboratory parameters of the PE group vs. controls, revealed that βhCG, hCG-H and hCG-H/β-hCG ratio were signifciantly higher in PE group compared to controls, in all comparisons, (P. value < 0.05). So as for, Serum uric acid, Blood urea, ALT and AST, all were signifciantly higher in PE group than controls. Serum creatinin appeared to be relatively higher in PE group than controls, but the difference did not reach the statistical signifciance (P. value >0.05), all these findings are demonstarted in (**Table 3**). To assess the validity hCG-H to β-hCG ratio in prediction of PE, we used the Receiver Operating Characteristics (ROC) Curve as classification analysis test. ROC analysis revealed that at different cutoff points for the hCG-H /βhCG ratio of 1.59, 1.90 and 2.70. The 1.9 cutoff point hac better performance than other two points; there were acceptable Sensitivity, Specificity, positive predictive value (PPV), negative predictive value (NPV) and Accuracy (**Figure 1 and Tble 4**).

Table 1. Comparison of maternal and gestational age in PE and control groups

Variable		Preeclampsia (n = 50)		Controls (n = 50)		P. value
		No.	%	No.	%	
Maternal Age (year)	< 20	24	48.0	28	56.0	0.545 ns
	20 - 29	20	40.0	16	32.0	
	30 - 39	6	12.0	6	12.0	
mean age		23.1 ± 4.9		22.6 ± 5.1		0.618 ns
Gestational age (week)	≤ 36 (preterm)	26	52.0	25	50.0	0.841 ns
	> 36 (Term)	24	48.0	25	50.0	

* ns : Chi-square test was not significant , P. value > 0.05

Table 2. Comparison of blood pressure and albumin in urine according of the studied groups

Variable		Preeclampsia (n = 50)		Controls (n = 50)		P. value
		No.	%	No.	%	
Blood pressure	Normal	2	4.0	47	94.0	< 0.001[S]
	Mild	22	44.0	3	6.0	
	Moderate to severe	26	52.0	0	0.00	
Albumin in urine	No	8	16.0	45	90.0	< 0.001[S]
	+	10	20.0	5	10.0	
	++	28	56.0	0	0.00	
	+++	4	8.0	0	0.00	

* Chi-square test was significant , P. value < 0.05

Table 3. Comparison of mean values of laboratory parameters of the studied groups

Variable	Preeclampsia	Controls	P. value*
	Mean ± SD	Mean ± SD	
βhCG (mlU/ml)	45.54 ± 34.44	17.05 ± 13.2	0.002 [S]
hCG-H (mlU/ml)	124.02 ± 41.8	13.9 ± 14.8	< 0.001 [S]
hCG-H/β-hCG ratio	4.68 ± 5.58	1.69 ± 1.81	0.03 [S]
Serum uric acid (mg/dl)	4.9 ± 1.0	3.6 ± 0.8	< 0.001[S]
Serum creatinine (mg/dl)	0.84 ± 0.12	0.8 ± 0.08	0.08 [ns]
Blood urea (mg/dl)	32.36 ± 7.8	29.3 ± 3.3	0.010 [S]
ALT (IU/L)	33.2 ± 28.1	16.7 ± 11.5	0.003[S]
AST (IU/L)	41.6 ± 36.1	17.1 ± 14	0.001[S]

*Independent sample t-test, S:Significant, NS:Not significant.

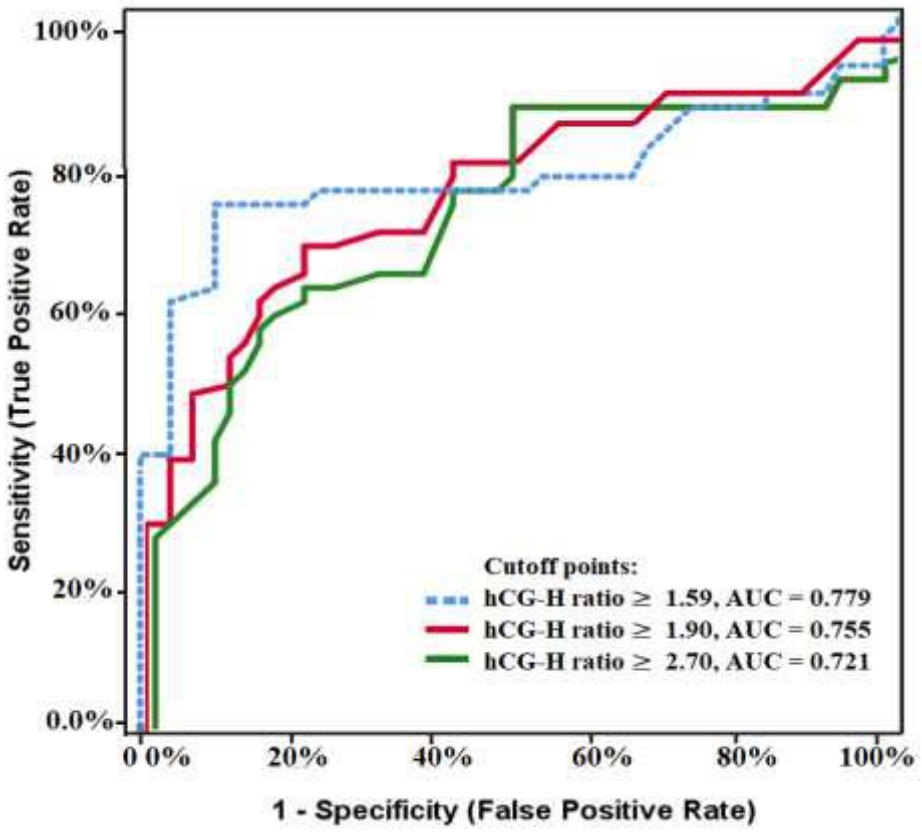

Figure 1. Receiver Operating Characteristics (ROC) Curve diagram for the validity of hCG-H ratio in prediction of Preeclampsia

Table 4. ROC coordinates for prediction of preeclampsia by hCG-H ratio.

Cutoff point	Sensitivity	Specificity	PPV	NPV	Accuracy
1.59	78.9 %	63.2 %	76.5 %	60.1 %	65.5 %
1.90	73.3 %	68.4 %	72.3 %	70.5 %	70.7 %
2.70	63.2 %	73.7 %	62.3 %	71.5 %	69.5 %

4. DISCUSSION

The preeclampsia is a systemic disease develops at early pregnancy responsible for high rates of co-morbidities and deaths (12). The screening of preeclampsia helps the physicians to plan complete monitoring and therapeutic schedule and prevention of complications (13). Pathology explanation of preeclampsia is based on impairment of invasion of cytotrophoblasts into the spiral arteries (14). Till now, specific activity of hyperglycosylated human chorionic gonadotropin in preeclampsia is not fully understood. Although this

finding, an obvious fact was observed that extravillous cytotrophoblasts stimulates hyperglycosylated human chorionic gonadotropin secretion at differentiation from proliferative cytotrophoblasts to invasive cytotrophoblasts in normal pregnancies and serum hyperglycosylated human chorionic gonadotropin predicts the invasion process of trophoblasts at 1st trimester (15). Decline in hyperglycosylated human chorionic gonadotropin levels at late pregnancy and mild preeclampsia might predict mild insufficient development of placenta (16). Our study showed that mean gestational age of pregnant women with PE was 33.5±2.1 weeks. Our study is the first national Iraqi study discussing the association of hyperglycosylated human chorionic gonadotropin percent to beta-HCG in preeclampsia at late pregnancy and it is noteworthy to mention that there is paucity in resources and researches in this subject. Present study showed that means hCG-H level of pregnant women with PE were significantly higher than mean hCG-H level of healthy controls (P<0.001, P=0.03, respectively) at late pregnancy and hence, we couldn't compare the level of hCG-H percent in early pregnancy to late pregnancy and proof its role in prediction of preeclampsia. The significant increase of means hCG-H level percent at late gestational ages may be attributed to factors related to increase the production from placenta secondary to placental ischemia in severe preeclampsia or decrease degradation in the kidney due to renal impairment in preeclampsia that lead to decrease the excretion of hCG-H or both. Current study found that cutoff hCG-H level of 1.9 mIU/ml had acceptable validity results (73.3% sensitivity, 68.4% specificity, 72.3% PPV, 70.5% NPV and accuracy 70.7%).

Our study showed that mean beta hCG of pregnant women with PE was significantly higher than beta hCG of healthy controls (p=0.002). This finding is consistent with results of Bayram et al study in Iraq on 80 women which found an elevation of beta hCG among women with pregnancy induced hypertension as compared to controls but in different groups divided according to trimesters and maternal ages (17). A prospective population-based cohort study on 7745 women carried out ,in Netherlands by Barjaktarovic et al, found a higher level of human chorionic gonadotropin in early pregnancy among women with high risk of preeclampsia and this level was also increased with higher severity of preeclampsia (18). The human chorionic gonadotropin is a hormone of pregnancy developed by trophoblast cells that organize the development of progesterone, implanting, growth of uterus and participated in immune system (19). The human chorionic gonadotropin is

important in regulating the placental development, angiogenesis and vasculogenesis through its role in vascular endothelial growth factors (20,21). Many authors found that increased or decreased levels of human chorionic gonadotropin are regarded as a predictor of preeclampsia (22, 23). Our study showed an association between preterm gestational age of pregnant women and preeclampsia (P=0.004). Similarly, Backes et al study in USA documented that prematurity is the common neonatal outcome of preeclampsia (24). Recent Chinese study on 185 women with preeclampsia, found that preterm preeclampsia is directly related to high risk of severe preeclampsia and neonatal morbidities more than term preeclampsia (25). Our study found that mean blood urea of pregnant women with PE was significantly higher than mean blood urea of healthy controls (P=0.01). This finding is similar to results of Müller-Deile et al study in Germany which reported higher blood urea level among women with preeclampsia (26).

Limitations, we involved pregnant women at gestational age between 32 weeks -40 weeks due to Covid 19 restriction at time of data collection that minimize the number of patients visiting the antenatal care unit in our province .Other limitations in our study are the limited time for data collection and excessive numbers of lost to follow up (LTFU) subjects especially those with early gestational ages that reduce the sample size and range of gestational ages

5. CONCLUSION

The hyperglycosylated human chorionic gonadotropin percent to beta-HCG were increased significantly in preeclampsia at late pregnancy.

Ethical Issues: All ethical issues were approved by the authors from the Iraqi Ministry of Health. Verbal and signed informed consents were obtained from all patients who included in the study during their first visit.

Conflict of interest: None

Source of funding: Authors declared no funding agency, or organization

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