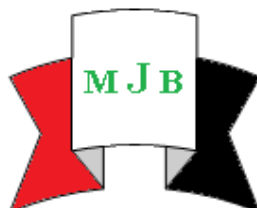


The Role of C-Reactive Protein and Visfatin in Pathogenesis of Preterm Labor in Al- Najaf City

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Abstract

Background: Visfatin is adipokine originally discovered as a pre- B-cell colony enhancing factor, is expressed by amniotic epithelium, cytotrophoblast, and deciduas and is over expressed when fetal membrane are exposed to mechanical stress or inflammatory stimuli.

Objective: To determine whether preterm labour is associated with changes in maternal plasma circulating C-reactive protein (CRP) and visfatin concentrations.

Study design: This is a case control study.

Setting: The department of Gynecology and Obstetrics in the college of medicine in Kufa University.

Materials and Method: The patients included in this study were divided into three groups control group who are preterm not in labor(50) and cases group who are preterm in labor(50) and full term group in labor(50). Parameters include age, gestational age, general urine examination, liver function test, renal function test, white blood count, hemoglobin, c-reactive protein (CRP), and visfatin.

Results: There is a significant increment in the level of C-reactive protein and visfatin between cases and control groups .

Conclusion: The visfatin and c-reactive protein may be involved in the pathogenesis of preterm labor.

Keywords: prterm labour (PTL), pathogenesis, C- reactive protein, Visfatin.

الخلاصة

الفسفاتين هو أديبوكين وهو يماثل بروتين و يعرف بالعامل المعزز لمستعمرات طليعة الخلية البائية و الذي يفرز عند تعرض الغشاء الجنيني لشدة خارجية أو أي محفز التهابي.

إن هدف هذه الدراسة هو معرفة اهمية قياس نسبة الفسفاتين والبروتين الفعال نوع سي لدى الحوامل اللاتي يعانين من ولادة مبكرة حيث أجريت الدراسة للمريضات اللواتي يراجعن صالة الولادة والعيادة الخارجية في قسم النسائية والتوليد في مستشفى الزهراء التعليمي للولادة والأطفال في مدينه النجف الاشرف و هي دراسة حاله سيطرة في قسم النسائية والتوليد في كليه الطب/جامعه الكوفة. حيث شملت الدراسة ١٥٠ مريضه قسمت الى ثلاث مجاميع الأولى تشمل خمسون امرأة حامل في حاله ولادة مبكرة والثانية خمسون أمراه في نفس عمر الحمل لكن ليس لديها ولادة والثالثة حامل في الشهر التاسع و لديها ولادة. التغيرات المثبتة تشمل العمر , وظائف الكبد, وظائف الكليتين, نسبة الدم, كريات الدم البيضاء, الفسفاتين والبروتين الفعال نوع سي حيث يتم سحب عينات الدم من الأم ومن الحبل السري و سحب كمية قليلة من السائل الامنيوسي بعد الولادة مباشرة وتم قياس نسبة الفسفاتين والبروتين الفعال سي. بينت هذه الدراسة زياده معنوية في نسبة الفسفاتين والبروتين الفعال سي لدى الحوامل اللواتي يعانين من ولادة مبكرة مما قد يعكس اهميته في حدوث حالات الولادة المبكرة.

Introduction

Preterm labor and delivery are among the most challenging obstetric complication encountered by the family physician, it is defined as delivery of baby before 37 completed week of pregnancy (1). The incidence of preterm birth in the develop world is 7-12%. their has been a small gradual rise in the incidence of preterm birth associated with assisted reproduction causing multiple pregnancies and an increased tendency to obstetric intervention. the rate of preterm birth prior to 32week has remained relatively stable at 1-2%(2).

There is compelling evidence that cytokine play a central role in the mechanism of infection include preterm labor.(3,4) . Interleukin 1 and tumor necrosis factor alpha produce by human deciduas in response to stimuli by bacterial product (5)stimulate prostaglandins production by amnion and deciduas(6) another cytokine increase was IL6,IL16 a granulocyte colony stimulating factor.(7).

C-reactive protein (CRP) is a sensitive marker of systemic inflammation and is primarily synthesized in hepatocytes in response to infection and tissue injury (8). Production of CRP is stimulated by the release of proinflammatory cytokines including interleukin-1, interleukin-6, and tumor necrosis factor-alpha. Although sometimes referred to as an acute-phase reactant, CRP accompanies both acute and chronic inflammatory disorders. Maternal concentrations of CRP have been studied as an aid to diagnose subclinical infection in pregnant women who experience preterm labor and premature rupture of membranes (9, 10).

Visfatin is(52KDa)cytokine that has been identified as a growth factor for early Bcell,termed pre-B cell colony enhancing factor(11). Visfatin expressed in visceral fat intuitively expressed in myometrium,placenta,all layer of human fetal membrane(12).Its gene is up-regulated in fetal membranes in response to mechanical stretch and term or preterm labor(13,14), parturition also cause increase

gene expression from myometrium,as well as the membrane, resulting in an increased protein in maternal serum(15) .

visfatin increase in a number of inflammatory condition including acute lung injury, colon cancer, sepsis, psoriasis, metabolic syndrome, rheumatoid arthritis also in chorioamnionitis. The mechanism of secretion is unknown however its exogenous application to explants of fetal membrane causes a number of pro-inflammatory modulators(TNF alpha,IL6,IL1beta,IL8) and enzymes to increase.(16) .

visfatin is anti –apoptotic for both amniotic epithelial and mesenchymal cell and neutrophils(17). Collectively these finding suggest that visfatin play role in inflammatory process in regulation of other pro inflammatory cytokines and in the preservation of immune cell.

The physiological role of visfatin in the inflammatory process of preterm labor, the presence of visfatin in amniotic fluid and its association with acute infection in humans. Thus, increased secretion of this protein from the fetal membrane, and possibly from intra-amniotic leukocytes in the presence of infection, may be an attempt to protect the epithelial cell from apoptosis(18).

The anti-apoptotic effect of visfatin on epithelial cell may have clinical implications.

Aim of Study

To determine whether preterm labour is associated with changes in maternal plasma circulating CRP and visfatin concentrations.

Materials and Methods

A case control study from the first of march to end of September 2013 include a total number of 150 pregnant women, 50 women preterm not in labor (depend on her gestational age before 37 completed weeks of pregnancy) and 50 women preterm in labor and 50 patient term pregnancy in labor . all these patient attending obstetric and gynecology

department in Al-Zahra Teaching hospital in Al-Najaf city /Iraq, were investigated for analysis of serum level of visfatin and c-reactive protein , history sheet was completed where date pertaining to age ,gravida ,parity,abortion,blood pressure, liver function test ,renal function test and hemoglobin.

A written consent was obtained from all qualified pregnant women volunteers after explaining the purpose of the study and the confidentiality of collected data and results.

The exclusion criteria include:

Medical disease (hypertension, diabetes mellitus, renal diseases and heart problem) and the presence of infection and antepartum hemorrhage.

The inclusion criteria:

included patients in the following groups: Normal pregnant women preterm not in labour as a control group, women with an episode of PTL and women who delivered at term.

Biochemical Investigation:

Blood samples were collected from participating women ,and cord blood samples were collected from umbilical cord after delivery .

Procedure:

The study was conducted to verify the changes of visfatin level in pregnancy to achieve this aim, the concentration of visfatin were determined using specific

and sensitive enzymes immunoassay . Visfatin enzyme immunoassay are based on the principle of competitive binding and were conducted according to recommendations of the manufacturer. Blood was drawn from vein using a sterile needle and syringe into an appropriate tube. the samples in plain tube were allowed to clot and is turbid and serum was separated by centrifugation for 10 min at 4000rpm into plain tubes and stored at -20 C until time of analysis.

Data handling

Statistical analysis was done by using SPSS(statistical package for social science) version 20 in which we use independent sample T-test to compare between two measurement data.

We set p value <0.05 as significant.

Results

The results of our study consists of 50 cases of preterm in labor and 50 controls(preterm not in labor) and 50 case of full term in labor. The age of cases range from 16-41 years old and the mean age of cases was 23.2 years while the age of control group range from 18-41 years old and the mean age of controls was 24.8 years with no significant difference between the two groups regarding their age ,gestational age ,hemoglobin, WBC, liver function and renal function as shown in table1.

Table(1) comparison between cases and controls regarding different parameters.

Parameter	Cases(50)(preterm in labour)	Controls(50)(preterm not in labour)	Full term in labour	P value
	Mean ±SD	Mean ±SD	Mean ±SD	
Age (years)	23.2±9.29536	24.8±6.71277	25.2±7.876	0.401
Gestational age(weeks)	32.08±1.82768	32.72±2.02071	38.23±1.89	0.006
Hemoglobin(g/dl)	10.6840±1.11270	10.9720±1.31212	10.894±1.143	0.372

WBC(9.8024±3.95658	10.4240±2.01084	9.78±2.89	0.481
SGOT(U/L)	16.4800±1.91918	15.4700±3.88823	16.37±2.32	0.763
SGPT(U/L)	13.1520±3.22391	12.5200±1.52850	14.11±2.86	0.171
RBS(mg/dl)	96.9000±12.94321	93.8200±10.12876	97.47±9.65	0.319
B.urea(mg/dl)	21.1000±2.07266	20.4000±2.15710	19.2±2.14	0.216
S.creatinine(mg/dl)	0.8000±0.07559	0.8140±0.11068	0.8±0.13	0.427

There was significant increment in the level of C-reactive protein and visfatin among cases in comparison with control group as shown in table number 2

Table(2) comparison between cases and controls regarding visfatin and CRP.

Parameter	Cases(50)(<i>preterm</i> in labour)	Controls(50)(<i>preterm</i> not in labour)	P value
	Mean ±SD	Mean ±SD	
CRP(mg/ml)	14.5200±6.97295	6.3600±1.43939	<0.001
B.visfatin(ng/ml)	57.2400±13.69189	29.7680±7.78333	<0.001

There was no significant changes in the level of visfatin in maternal blood, amniotic fluid and cord blood when compared between cases and full term group but there is significant increment of CRP in preterm in labour cases when compared with term in labour women which shown in table number 3.

Table(3) comparison between cases (*preterm*) and full term delivery of CRP, B.visfatin , AF and cord B. visfatin

Parameter	Cases(<i>preterm</i> in labor)	full term in labour	P value
	Mean ±SD	Mean ±SD	
CRP	14.5200±6.97295	6.62±1.27	<0.001
B.visfatin	57.2400±13.69189	61.3±8.24	0.275
AF visfatin	65.708±12.94	65.33±14.2	0.904

Cord B.visfatin	34.15±9.37	31.94±9.6	0.314
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Discussion

Result obtained from this study show significant increase in concentration of visfatin and c- reactive protein in preterm labor when compared to the control group. this suggest a possible effect of visfatin and c-reactive protein in development and pathogenesis of preterm labor.

Up to now ,several clinical and biochemical markers have been reported to be associated with preterm delivery. increased level of maternal serum CRP are significantly associated with increased risk of preterm labor(19).

CRP is sensitive inflammatory marker that remains stable in maternal serum(20). A study done by Ruben Ovadia(21) on 850 women preterm pregnancy showed marked increase in level of c- reactive protein in association with periodontal disease lead to preterm delivery, and Hvilsom GB et al report showed increase concentration of c-reactive protein by about two fold in preterm labor(22). Gheizzi et al study support the hypothesis that subclinical fetal inflammation response might occur very early during pregnancy in fetus, who will experience preterm delivery(23).

Immunoreactive visfatin is a physiologic constituent of the amniotic fluid. the median amniotic fluid concentration of visfatin increase with advancing gestational age(18,24). Visfatin is a novel adipokine(25) that has been previously identified as a growth factor for early B – cell, termed as pre-Bcell colony –enhancing factor(26)Its gene mapped on the long arm of chromosome7(10),In human visfatin produce by visceral adipose tissue it expressed in large amount in bone marrow, liver, muscle, heart, lung, kidney, placenta, monocytes,and neutrophils.(27)

There is evidence to suggest that visfatin has at least three major features: - immunoregulatory, metabolic and intracellular phosphoribosyl tranferase activity. The increase in the median

concentration of amniotic fluid visfatin with advancing gestation and the positive correlation with gestational age support the aforementioned report regarding the association between fetal membrane stretching and increased expression and secretion of visfatin. In addition, visfatin has anti-apoptotic properties, and can exert its effect on amniotic epithelial cell, fibroblast and neutrophils, this is confirmed by Sethi Jk et al (28) how take 60 women and study the role of visfatin in labor, it is tempting to postulate that the physiological increase of visfatin in amniotic fluid with advancing pregnancy aims to protect the fetal membrane from apoptosis and thus , prevent preterm PROM. Alternatively, given the unique combination of immunoregulatory and anti-apoptotic properties of visfatin, it can be argued that its concentration increases in the presence of infection of amniotic cavity as a defense mechanism against the inflammatory process or to help sustain neutrophils in the amniotic fluid. Finally Martha Lappas collect data indicate that visfatin activates pro-inflammatory cytokine release and phospholipid metabolism in human placenta via activation of the NF-κB pathway. Thus, visfatin represents a novel cytokine linked to the events of human labour initiation(29).

Conclusion

The visfatin and c-reactive protein may be associated in the pathogenesis of preterm labor.

Recommendation

- 1- Further study may be required for larger number of patients.
- 2- Study the level of c-reactive protein and visfatin in different gestational ages.
- 3- To evaluate the predictive significance of C- reactive protein and visfatin in spontaneous preterm delivery.

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