Original Article

Evaluation of Neurokinin B in Severe Preeclamptic Pregnancies & Their Impact on Pregnancy Outcomes.

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Abstract:

Aim of the work: The aim of the present study is to evaluate serum neurokinin b level in third trimester of pregnancy in women with severe pre-eclampsia in comparison to a control group of pregnant females and its impact on pregnancy outcomes. **Methods & Patients** The study was done on 78 pregnant females in Main University Hospital in Alexandria. The patients were categorized into two groups 38 females each: Group 1: 38 female as control, Group 2:38 female with severe preeclampsia, both are in their third trimester >34 weeks of gestation, primigravidas, didn't receive antihypertensive medications before. Obstetric, menstrual & medical histories were taken from all patients. Complete general examination was done & investigations were done to both mothers and to their foetuses pre & postoperative. Preoperative investigations & assessments related to the mother include :mean blood pressure, urine output per day, complete blood picture, serum glucose level, liver function tests & kidney function tests, whereas for postoperative investigations & assessments were the same as preoperative plus monitoring blood loss, postpartum complications, mood of delivery & need for icu. Preoperative investigations & assessments related to the foetus was ultrasonography to assess viability, gestational age & foetal weight; whereas for postoperative investigations Apgar scoring, foetal weight & need for icu were assessed. For Neurokinin B assay method used was Enzyme-Linked Immunosorbent Assay Kit where samples were aspirated on a serum separator vacutainers and allowed to clot for 2 hours at room temperature. Centrifugation was done for each sample for 20 minutes at approximately 1000xg and samples were stored at -20 degree Celsius till time of assay. **Results & Conclusions:** From the present study we concluded that Neurokinin b serum levels are higher in preeclamptic females in comparison to normotensive ones. Neurokinin b levels play a role in the pathophysiology of preeclampsia. Preeclamptic females with high levels of neurokinin b had good pregnancy outcomes with fewer complications.

Introduction:

Preeclampsia is a major cause of maternal mortality & morbidity, perinatal deaths, preterm births, & intrauterine growth retardation, especially in developing countries.[1,2] It occurs in 3 - 4% of all pregnancies worldwide, and in 10% before 34 weeks of gestation.[3]

The precise origin of preeclampsia is multifactorial and includes a scenario of required steps.[4] It often affects young & nulliparous women, whereas older women are at greater risk for chronic hypertension with superimposed hypertension. Also, the incidence is markedly influenced by race, ethnicity & by genetic predisposition. Other factors include environmental, socioeconomic & even seasonal influences.[5]

Preeclampsia was defined as systolic blood pressure ≥ 140 mm hg &/or diastolic blood pressure ≥ 90 mm hg, measured on at least 2 consecutive occasions, at least 4 to 6 hours apart. Furthermore, first presentation had to be after 20th week of gestation, in a previously normotensive women, & coupled with significant proteinuria (>300 mg/dl with 24 hour urinary collection; +1, +2 on a qualitative dipstick examination).[6]

Mild preeclampsia is defined as the presence of 1 of the following symptoms or signs in the presence of preeclampsia.

AS for severe preeclampsia, it is defined as the presence of 1 of the following symptoms & signs in the presence of preeclampsia:

* Systolic blood pressure of 160 mm hg or higher or Diastolic blood pressure of 110 mm hg or higher on 2 occasions at least 6 hours apart.
* Proteinuria of more than 5 gm in a 24 hour collection or more than +3 on 2 random urine samples at least 4 hours apart.
* Pulmonary edema or cyanosis.
* Oliguria (<400 ml in 24 hour).
* Persistent headaches.
* Epigastric pain &/or impaired liver functions.
* Thrombocytopenia.

Preeclampsia affects approximately 3 to 10 percent in nulliparous populations while it's less than that in multiparous females,[7] However the risk for stillbirth was more likely in multiparous compared to nulliparous females.[8]

Risk factors for preeclampsia include: Gestational age between 32 & 36 weeks of gestation increased the risk from 1.1 to 1.8 %,[9] Parity where nulliparity triples the risk for preeclampsia.[10] Multiple pregnancy where pregnant women with twins triples the risk, [11] Maternal Age ;women aged >40 years had approaching twice the risk,[12] Race; the incidence of preeclampsia is 1.8 % among white females & 3 % among black ones,[13] women with previous preeclampsia have seven times increased risk for recurrence in subsequent pregnancies.[14] Obesity increase the risk from 4.3% in women with BMI <20 kg/m^2 to 13.3% in women with BMI >35kg/m^2 ,[15] a Family history nearly triples the risk,[16] Vitamin D deficiency increase the risk of preeclampsia & foetal growth restriction[17] & Smoking where current cigarette smokers during pregnancy seem to have a decreased risk. [18]

Numerous factors currently are considered to be implicated in the development of preeclampsia which are maternal immunologic intolerance, abnormal placental implantation, genetic factors, angiogenic factors, oxidative stress & inflammation, renin angiotensin system, nutritional & environmental factors & additional factors in preeclampsia. [19]

Pathophysiology of preeclampsia include various body systems including; Cardiovascular system where hypertension present due to marked vasoconstriction causing both cardiac output & arterial compliance to be reduced.[20] The Kidney where renal plasma flow & glomerular filtration rate are both decreased by about 25%.[21] The placenta where there is failure of normal trophoblastic invasion of spiral arteries leading to hypoxia & release of angiogenic proteins that initiate preeclampsia.[22] The Brain where its effect is referred to as the posterior reversible encephalopathy syndrome [23] & the Liver with the Coagulation abnormalities where there is usually mild thrombocytopenia as the most commonly detected abnormality, increased platelet activation & size leading to hypercoagulability,[19] aspartate aminotransferase & lactate dehydrogenase are also elevated [24] & gross hepatic changes were reported to be in the form of hepatic hemorrhage from areas of infarction.[24, 25]

Prognosis of preeclampsia & eclampsia tend to worsen with the following conditions: [26]

* Systemic endothelial dysfunction.
* Vasospasm & small vessel thrombosis leading to tissue & organ ischemia.
* CNS events, such as seizures, strokes, and hemorrhage.
* Acute tubular necrosis.
* Coagulopathies.
* Placental abruption in the mother.

AS for the, Prevention of preeclampsia; low dose aspirin may reduce incidence by 10 %.[27] For treatment of preeclampsia many drugs can be used including;[28,29] Methyldopa which is considered the drug of choice & safety after first trimester is well documented, Labetalol given in 200-1200mg/day in 2-3 divided doses, Nifidipine which is a calcium channel blocker given in 30-120 mg/day of a slow release preparation & has synergistic effect with magnesium sulfate, Hydralazine which is useful only in combination with sympatholytic agents given in 50-300 mg/day in 2-4 divided doses, Beta blockers but may

cause growth restriction when given in first or second trimester especially atenolol & ACE inhibitors with AT 1 receptor antagonists,[30] are both contraindicated cause of the major anomalies associated which may be fatal.

Magnesium Sulphate is the anticonvulsant of choice for both prevention & treatment of eclampsia. [31] The exact mechanism of action is the blockade of N-Methyl D-Aspartate receptors involved in seizure genesis or calcium channel blocking preventing cerebral vasospasm.[32] Side effects include[33]; hypotension, facial flushing, visual disturbances, chest pain, nasal stiffness, circulatory collapse, gastrointestinal upset, urine retention, magnesium toxicity. Contraindications & precautions include; [33] as it is excreted by the kidney should be used with caution in those with impaired renal functions, with those using digitalis/cardiac glycosides & with CNS depressants as it may result in an enhanced CNS depression too. Prophylactic dose; loading dose at first using 10 ml vial, prepare 4 gram of 50 % magnesium sulfate given at rate 32 ml per hour for 15 minutes, where the maintenance dose prepare 50 ml of 50 % magnesium sulfate & administer at a rate of 1 gm/hr until at least 24 hours post birth/delivery. [32]

As it is now believed, preeclampsia is hidden in the placenta; researchers are directed to novel peptide genes in this organ. Using mRNA fingerprinting[34] & human genomic database,[35] they found nine matches that showed high similarity to the bovine neurokinin b precursor.[36] Neurokinin b mRNA expression was found to be restricted to the outer syncytiotrophoblast of the human placenta, in an ideal position to be secreted into the maternal serum.[37]

From all candidates, neurokinin b which is a member of the tachykinin family,[38] appeared to be the most promising as a potential marker & factor to cause pre-eclampsia. The placenta was found to express unusually high levels of TAC3 that encodes neurokinin b,[39] a gene previously believed not to be expressed in any peripheral tissue,[40] but to the outer syncytiotrophoblast.[41] Elevated expression levels of TAC3 are found to be significantly higher in preeclamptic placenta at term when compared to controls.[42, 43]

IN terms of neurokinin b binds & activated the neurokinin 3 receptor, evoking vasoconstriction in the mesenteric & portal veins, & increasing heart rate; indicative of pressor activity. Theses cardiovascular effects of neurokinin b culminate in the clinical manifestations of preeclampsia. Also it causes increase in the blood pressure & damage to the kidneys & liver observed in preeclampsia. At very elevated concentrations NK1 receptors on platlets may be affected, eventually causing thrombocytopenia.[44]

Other studies showed that neurokinin b acted as a dilator in the placental vasculature.[45,46] Where this effect was found to be initiated solely through the neurokinin 1 receptor. The NK1 receptor is associated with vasodilatory responses,[41] and evidence has shown that the vasoconstrictive NK3 receptor is either absent or expressed at extremely low levels in the human placenta at term when compared to NK1 & NK2 receptors.[42,45] This would advocate a mechanism in the human placenta, whereby high NKB levels induce placental vasodilatation via NK1 receptor to maintain low placental resistance. Nonetheless, preeclampsia is typically associated with maternal vasoconstrictive responses and we proposed that activation of NK3 receptors on the venous side of the maternal circulation could be responsible for hypertension that develops during pre-ecalampsia.[39,41] It was also found that there's a link between elevated NKB level in women with preeclampsia in the third trimester and haemodynamic adaptation via nitric oxide production. [47]

Results:

As regard to patient's SBP, in the control group it ranged between (110 – 120) mmHg

with mean ± S.D (117.37±4.463) mmHg while in severe preeclampsia group it ranged between (160 – 200) mmHg with mean ± S.D (174±11.503) mmHg. There was statistically significant difference between the studied groups where P=0.000 (P significant as P<0.05). **(Table I)**

As regard to patient's DBP, in the control Group it ranged between (70 – 80) mmHg with mean ± S.D (73.68±4.889) mmHg while in severe preeclampsia Group it ranged between (110–120) with mean±S.D (110.75±2.667) mmHg. There was statistically significant difference between the studied groups where P< 0.001.

As regard to patient's MAP, in the control Group it ranged (83.33 – 93.33) with mean ± S.D. 88.246 ± 3.171 while in severe preeclampsia Group it ranged (126.67 – 140) with mean ± S.D. 131.833±3.77. There was statistically significant difference between the studied groups where P=0.000 (P significant as P<0.05). **(Table III)**

As regard to patient's MAP, in the control Group it ranged (83.33 – 93.33) with mean ± S.D. 88.246±3.171 while in severe preeclampsia Group it ranged (126.67 – 140) with mean ± S.D. 131.833±3.77. There was statistically significant difference between the studied groups where P=0.000 (P significant as P<0.05). **(Table IV)**

As regard to patient's EFW, in the control Group it ranged between (2800 – 3400) with mean ± S.D. (3131.58 ± 141.034) while in severe preeclampsia Group it ranged between (2000 – 2910) with mean ± S.D (2444.38 ± 254.718). There was statistically significant difference between the studied groups where P=0.000 (P significant as P<0.05). **(Table V)**

As regard to patient's AFI, in the control Group it ranged between (9.4 – 14) with mean ± S.D. 11.668 ± 1.149 while in severe preeclampsia Group it ranged between 7 – 12 with mean ± S.D. 10.385 ± 1.638. There was statistically significant difference between the studied groups where P=0.000 (P significant as P<0.05). **(Table VI)**

As regard to patient's Neurokinin B, in the control Group it ranged between (0.8 – 50) with mean ± S.D. (20.388 ± 17.417) while in severe preeclampsia Group it ranged between (0.1 – 150) with mean ± S.D. (50.699 ± 55.215). There was statistically significant difference between the studied groups where P<0.001 (P significant as P<0.05). **(Table VII)**

As regard to patient's complication, in the control Group all patients had no complication while in severe preeclampsia Group 31(77.5%) out of the patients had no complication and 9(22.5) out of the patients had complication (all of them had NICU while 6 of the patients had IUGR and ICU and 3 patients had eclampsia, finally 1 of the patients had HEIIP). There was statistically significant difference between the studied groups where P=0.000 (P significant as P<0.05). **(Table VIII)**

Table (I): Comparison between the two groups regarded to SBP

SBP	Control Group (n=38)	Severe Preeclampsia Group (n=38)	Total
Min.	110	160	110
Max.	120	200	200
Mean	117.37	174.00	146.41
S.D	4.463	11.503	29.803
P Value	<0.001*		

Table (II): Comparison between the two groups regarded to DBP

DBP (mmHg)	Control Group (n=38)	Severe Preeclampsia Group (n=38)	Total
Min.	70	110	70
Max.	80	120	120
Mean	73.68	110.75	92.69
S.D	4.889	2.667	19.047
P Value	<0.001*		

Table (III): Comparison between the two groups regarded to MAP

MAP	Control Group (n=38)	Severe Preeclampsia Group(n=38)	Total
Min.	83.33	126.67	83.33
Max.	93.33	140.00	140.00
Mean	88.246	131.833	110.598
S.D	3.171	3.770	22.200
P Value	<0.001*		

Table (IV): Comparison between the two groups regarded to MAP

MAP	Control Group (n=38)	Severe Preeclampsia Group (n=38)	Total
Min.	83.33	126.67	83.33
Max.	93.33	140.00	140.00
Mean	88.246	131.833	110.598
S.D	3.171	3.770	22.200
P Value	<0.001*		

Table (V): Comparison between the two groups regarded to EFW

EFW	Control Group(n=38)	Severe Preeclampsia Group (n=38)	Total
Min.	2800	2000	2000
Max.	3400	2910	3400
Mean	3131.58	2444.38	2779.17
S.D	141.034	254.718	402.414
P Value	<0.001*		

Table (VI): Comparison between the two groups regarded to AFI

AFI	Control Group(n=38)	Severe Preeclampsia Group(n=38)	Total
Min.	9.400	7.000	7.000
Max.	14.000	12.000	14.000
Mean	11.668	10.385	11.010
S.D	1.149	1.638	1.553
P Value	< 0.001*		

Table (VII): Comparison between the two groups regarded to Neurokinin B

NKB	Control Group (n=38)	Severe Preeclampsia Group (n=38)	Total
Min.	0.800	0.100	0.800
Max.	50.000	150.000	150.000
Mean	20.388	50.699	35.932
S.D	17.417	55.215	43.845
P Value	<0.001*		

Table (VIII): Comparison between the two groups regarded to complication

Complications	Control Group (n=38)		Severe Preeclampsia Group (n=38)		Total	
	No	%	No	%	No	%
No	38	100	31	77.5	69	88.5
Yes	0	0	9	22.5	9	11.5
IUGR	0	0	6	15	6	7.7
NICU	0	0	9	22.5	9	11.5
ICU	0	0	6	15	6	7.7
HEIIP	0	0	1	2.5	1	1.3
Eclampsia	0	0	3	7.5	3	3.8
P Value	<0.001*					

Discussion:

Preeclampsia & eclampsia may occur in as many as 8 % of pregnancies and remain a leading cause of maternal and foetal morbidity and mortality.[48] The most recent official report from the UK Center for Maternal and Child Enquiries (CMACE) ranked preeclampsia as the second most common direct cause of maternal death.[49] Several theories have been advocated and by far the most compelling evidence that placenta holds the key.[50,51]

Neurokinin B is amongst these markers, which is a neuropeptide of the tachykinin family, and is expressed in the outer syncytiotrophoblast of the placenta. Detectable plasma concentrations in pregnant women were observed as early as 9 weeks. It is postulated that neurokinin B binds & activates the neurokinin 3 receptor, evoking vasoconstriction in the mesenteric & portal veins & heart rate: all factors indicative of pressor activity. It now seems probable that the vasoactive properties of neurokinin B are involved in the clinical manifestations of preeclampsia. [52]

In the presenting study we investigated the serum level of neurokinin b in 38 normal pregnant females (Group 1) in comparison to 38 pregnant females with severe preeclampsia (Group 2).

The aim of the present study was to evaluate serum neurokinin B level in pregnant females with severe preeclampsia in third trimester and to see its impact on pregnancy outcomes.

Both groups of pregnant females included in the study were investigated through analyzed detailed patient history and clinical systemic and local examination.

AS regards the laboratory investigations (AST, ALT and Haemoglobin level) there was no statistically significant difference between the 2 groups.

The results of our study showed that there was a significant increase in the serum level of neurokinin b in the preeclamptic group compared to control group by using

Enzyme-Linked immunosorbent (ELISA) Assay kit for neurokinin b (P<0.001)

Results of our study coincides with D'Anna et al[53] in a prospective randomized study that involved a total of 90 pregnant women. Thirty had a gestation complicated by preeclampsia and 30 by isolated IUGR; the other 30 were controls. In all patients neurokinin B plasma levels was measured. Neurokinin B blood samples were taken at 35 weeks of gestation and at term. Results showed that neurokinin B plasma levels in the preeclamptic and IUGR groups were significantly higher than controls.

Moreover, Geissbuehler V et al [54] in a study determined the levels of neurokinin B in the plasma of South African colored pregnant women with and without preeclampsia and correlated the results with clinical data. Additionally, the peptid radioimmunoassay (RIA) and the peptid enzyme immunoassay (EIA) methods were compared in the determination of neurokinin B levels, using 58 samples from patients with preeclampsia and 62 healthy pregnant women. Clinical data were gathered using questionnaires were 58 patient samples were tested by both RIA and EIA. The mean neurokinin B concentration in the preeclamptic group was significantly higher than in the control group.

Also, our results were in agreement with Geissbuehler V et al. [55] who in a prospective randomized study measured neurokinin B levels in pregnant females with and without preeclampsia in the third trimester. Third trimester plasma neurokinin B levels were determined by enzyme-linked immunosorbent assay technique (EIA) in 72 pregnant females with preeclampsia and in 94 healthy female. The EIA results were then correlated with clinical data. Results showed that the mean neurokinin B concentration in the preeclamptic group was significantly higher than in control group (P<0.001)

Furthermore, our results were in accordance with Zulfikaroglu E et al.[56] who in a prospective randomized study involved 22 preeclamptic and normotensive female, measured the peripheral and umbilical cord blood neurokinin B levels by radioimmunoassay. The neurokinin B levels in women with preeclampsia were 0.70 nmol/L in peripheral blood and 1.92 nmol/L in umbilical cord blood. In normotensive pregnant women, neurokinin B levels were 0.43 nmol/L and 0.14 respectively. Significantly higher levels of neurokinin B in preeclamptic females compared to normotensive ones in umbilical cord blood suggested that neurokinin B enters both fetal and maternal circulation and may modulate fetoplacental hemodynamics.

Again, Li ZM et al.[57] in a study that involved 22 women, who received antenatal examination in the Department of Obstetrics and Gynecology of Union Hospital of Tongji Medical College in Huazhong University of Science and Technology from March to July in 2005, including 12 gestational hypertension (gestational hypertension group) and 10 preeclamptic female (preeclamptic group); 22 normal pregnant women in the same period were served as control. At different gestational weeks, maternal plasma levels of neurokinin B in the three groups were detected by enzyme-linked immunoassay technique, the expression and location of neurokinin B in placenta were examined by immunohistochemical SP, and mRNA expressions of neurokinin B in placenta were measured with RT-PCR method.

There was significant difference between preeclamptic group and control group (P<0.05), while there was no significant difference between gestational hypertension group and control group (P>0.05).The expressions of neurokinin B in placenta of preeclamptic group were significantly higher than that in control group, with a significant difference between the two groups (P<0.05).

Also, Liu Y et al.[58] conducted a prospective randomized study that involved a total of 60 women in the third trimester of pregnancy, 40 women with preeclampsia (study patients) and 20 normotensive women (healthy controls). They were divided into three groups: the 20 normotensive pregnant women (Group 1); 20 women with mild preeclampsia (Group 2); 20 women with severe preeclampsia (Group 3). The plasma levels of neurokinin B were significantly higher in women with mild or severe preeclampsia (p50.01 for both groups) compared with controls.

Our results were in agreement with D'Anna et al[53] who in a randomized study,

showed a positive correlation between neurokinin B plasma levels and systolic blood pressure (r=0.719, p=0.029) and with diastolic blood pressure (r=0.058, p=0.94)

Our study was done using a sample size of 76 pregnant primigravidae females. First group is composed of 38 female (control group); their age range was from 18 to 30 years old , gestational age from 38 to 40 weeks of gestation, their systolic blood pressure range from 110 to 120 mmHg, their diastolic blood pressure range from 70 to 80 mmHg , with ultrasound follow up their mean estimated foetal weight was around 3130 gms while their mean amniotic fluid index was around 11.5 ml which indicates good pregnancy outcomes where no complications were found in these 38 pregnant females who had normal vaginal delivery with healthy babies. The mean value of serum neurokinin B measured before labour was around 20.4 pg/ml in this group.

While the other group of 38 preeclamptic pregnant females ; have their age range from 24 to 32 years old, at 35 to 39 weeks of gestation , their systolic blood pressure range from 160 to 200 mmHg with mean value around 174 mmHg , their diastolic blood pressure range from 110 to 120 mmHg with mean value around 111 mmHg, with ultrasound follow up the estimated foetal weight of their fetuses range from 2000 to 2910 grams with mean value around 2444 grams and the mean value of amniotic fluid index was around 10.39 ml according to gestational age. The mean value of serum neurokinin B of this group was around 50.7 pg/ml, where 31 patients of them suffered no complications with follow up done before and after delivery with their serum level of neurokinin B high or around 50.7 pg/ml.

While 7 patients out of these 38 preeclamptic females had their babies admitted to neonatal intensive care unit with their apgar scoring at 5 minutes range from 6-7 while at 10 minutes were all above 8 and in good condition, 6 out of which had intrauterine growth restriction with low birth weight and their estimated foetal weight and amniotic fluid index below normal value for their gestational age detected during their follow up.

Three females out of these 7 preeclamptic females had eclampsia and one had HELLP syndrome which was detected after investigations was done. Serum level of neurokinin B of these 7 females was found to be low or away from the mean value for this group which was equal 50.7 pg/ml.

After our study, we concluded that there is positive significant correlation between the serum level of neurokinin B with the age, systolic blood pressure and diastolic blood pressure, while there is negative correlation between serum level of neurokinin B with the gestational age and the risk of complications including (HELLP, eclampsia, IUGR, need for NICU and ICU admission).

Conclusions:

1. Neurokinin B blood levels are higher in preeclamptic women compared to normotensive women.
2. There is positive significant correlation between neurokinin B and age,SBP,DBP while there is negative correlation between neurokinin B and gestational age & complications.
3. Neurokinin B may play a role in the pathophysiology of preeclampsia.
4. Preeclamptic patients with high levels of neurokinin B had good pregnancy outcomes.
5. Preeclamptic patients with high neurokinin B serum levels have less risk of having intrauterine gross restricted foetuses and need for neonatal intensive care unit.
6. Preeclamptic patients with high neurokinin B serum levels have less risk for developing HELLP syndrome, eclampsia and other systemic complications.

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