

Determining Visfatin / NAMPT Serum Levels by ELISA Technique, in Pregant Women with Preeclampsia

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Preeclampsia is a syndrome that affects approximately 2-8% of all pregnancies [1] and is closely linked to a particularly elevated inflammatory response, its presence resulting in high maternal and neonatal mortality. Several adipokins have been found to be involved in this process. Visfatin is a cytokine that exerts its effects especially on metabolism and immunity. In this study we determined the serum profile of Visfatin using the ELISA technique, to the 40 patients diagnosed with preeclampsia and we compared it to 16 pregnant patients without complications. Patients with preeclampsia had lower, but statistically not significant plasma levels compared to those in the control group, 11.04 ± 7.14 (3.57-17.97) vs 8.88 ± 7.91 (1.37-17.85). The lowest Visfatin levels have been recorded in the most severe form of preeclampsia. Visfatin does not significantly influence the birth weight of the fetuses ($p = 0.08$).

Keywords: preeclampsia, inflammation, visfatin /NAMPT

It is well known that during pregnancy there is an intense inflammatory condition. Recent studies have shown that the inflammatory status is much exacerbated in women with preeclampsia. In addition, stimulation of neo-vascularization processes plays an important role in the occurrence of hypertension, endothelial dysfunction and ultimately leads to foetal hypotrophy and preterm birth [2]. Trophoblastic cells mediate inflammation through a series of mediators such as interleukin 1-beta, 2, 4, 6, 8, 10, 12, 18, TGF beta 1 (transforming growth factor), INF-gamma interferon gamma IP-10 (inducible protein 10); tumour necrosis factor-alpha (TNF- α), MCP-1 (monocyte chemotactic protein), ICAM-1 (intercellular adhesion molecule), vascular adhesion molecule) [3].

Visfatin, known to be a pre-B cell colony-enhancing factor, is secreted by the perivascular fat and macrophages, and is involved in glucose homeostasis [4]. The pleiotropic effects of visfatin are expressed in a series of physiological and pathophysiological processes, changes in its homeostasis being related to obesity, type II diabetes, sepsis, acute pneumopathies and various gestational complications, especially preeclampsia. Compared to the control groups, studies have demonstrated different outcomes (elevated, low or unchanged levels) of serum visfatin in patients with type 2 diabetes, obesity, gestational diabetes [5]. Defining the role visfatin plays in these pathological conditions is an important research topic. The reason for the increased visfatin level in preeclampsia has not been fully elucidated, but it appears that IL-6 and TNF- α down-regulates the expression of visfatin in perivascular adipose tissue.

Visfatin also has a nicotinamide phosphoribosyl transferase (NAMPT) like activity, so these enzymes are responsible for NAD⁺ levels (nicotinamide adenine dinucleotide) [6]. NAD⁺ has an especially important effect on intracellular redox reactions (fig. 1). The latest studies have demonstrated that mammalian cells possess a family

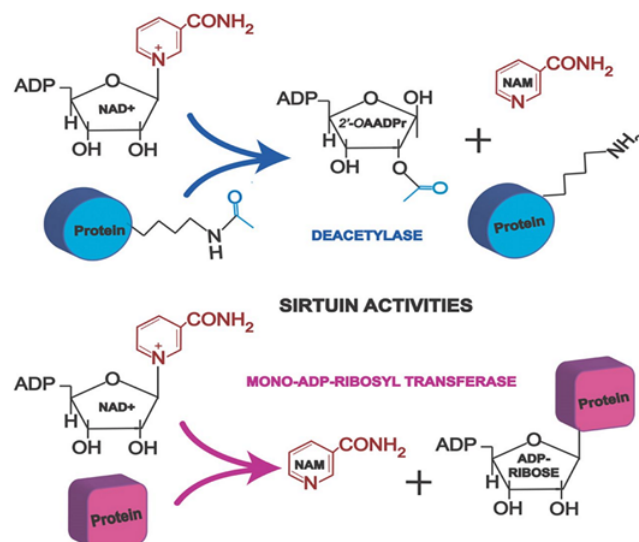


Fig. 1.Sirtuin enzymatic activities: Sirtuins are NAD⁺-dependent deacetylases and mono-ADP-ribosyltransferases that regulate a wide array of proteins involved in metabolism and cell survival.

The α -acetyl lysine residues of the target protein serve as substrates for sirtuin deacetylation, which generate 2'-OAAADPr as a by-product. NAM, nicotinamide [8]

of NAD⁺ dependent deacetylases, which are included in the *SIRTUIN* family. By implicating them in histone deacetylation and methylation, they modulate DNA transcription, and therefore they have been found to be involved in cell differentiation, cell senescence, and tumour proliferation [7]. Thus, the increase in NAMPT concentration results in an increase in NAD⁺ and SIRT1 activity. This promotes cellular dysfunction, with the occurrence of endothelial dysfunction that appears to be one of the mechanisms involved in preeclampsia.

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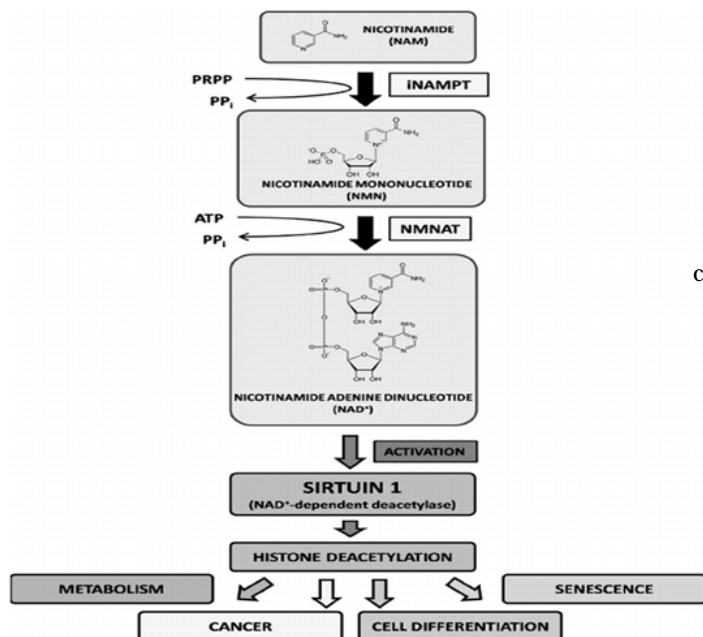


Fig. 2. Nicotinamide adenine dinucleotide biosynthesis. Nicotinamide phosphoribosyl transferase is the enzyme that catalyzes the transformation of nicotinamide mononucleotides into nicotinamide dinucleotides in the mammalian organism. [9]

Experimental part

We conducted a study on 56 pregnant patients:

40 diagnosed with moderate form preeclampsia (30 patients) and severe preeclampsia (10 patients),

16 control subjects - represented by healthy pregnant women, the blood being harvested after week 20 of pregnancy

Preeclampsia was defined by TA > 140 / 90 mmHg after 20 weeks after the last menstruation, accompanied by proteinuria > 0.3g / 24h. The study excluded patients with gestational diabetes, type 2 diabetes mellitus, polyhydramnios, chronic kidney disease, premature membrane rupture. The serum of these patients was analyzed after being harvested, centrifuged and frozen at -80 degrees, until it was determined.

VISFATIN / NAMPT determination technique

Visfatin was determined using the ELISA technique: a monoclonal antibody specific for NAMPT has been precoated into 96-well microtiter plate. Standard and samples were pipeted into the wells; NAMPT was recognized by the addition of a purified polyclonal antibody specific for NAMPT. Then HRP conjugated rabbit antibody IgG was added, and after a final washing, peroxidase activity is quantified using a 3,3',5,5'-tetramethylbenzidine

substrate. Then we measured the intensity of the colour at 450nm after acidification. The intensity was directly proportional to the concentration of NAMPT in the samples. NAMPT levels range in serum from healthy donors from 0.2 to >1.5ng/mL.

Statistical analysis

Analyses were run in IBM SPSS 24. Significance was considered at p-value < 0.05. Data was first qualitatively evaluated. Frequencies and percents were computed for nominal and ordinal data. Normality was assessed for scale variables. Descriptive were presented in the form of mean ± standard deviation or median (IQR in the form Q1- Q3). Due to the fact that none of these variables proved to be normally distributed, comparisons were made using non-parametric tests. Depending on the type of comparisons, we employed the Mann-Whitney test, Wilcoxon, Kruskal-Wallis and so on. Regression analyses were conducted in the binary logistic and ordinal form. Transformations were applied on variables in order to standardize them.

Results and discussions

Table 1 shows that significant differences between the control group and the patients one are to be found in the case of birth age (p-value = 0.005), systolic and diastolic

Variable	Preeclampsia		p-value
	No	Yes	
Week of pregnancy	38.73 ± 0.46 39 (38-39)	36.38 ± 3.26 38 (34-39)	0.005
systolic TA (mmHg)	111.33 ± 8.12 110 (105-120)	153.1 ± 11.79 150 (140-160)	0.000
Diastolic TA(mmHg)	61 ± 4.7 60 (60-60)	99.31 ± 7.54 100 (90-100)	0.000
Gestation period			0.126
Trim. I	8 (50%)	18 (45%)	
Trim. II	3 (18.8%)	17 (42.5%)	
Trim. III	5 (31.2%)	5 (12.5%)	
Number of previous births	1.67 ± 0.9 1 (1-2)	1.28 ± 0.51 1 (1-2)	0.055
Newborn weight (g)	3254.67 ± 289.23 3300 (3000-3500)	2418.46 ± 652.35 2600 (1800-2900)	0.000
Apgar index	9.53 ± 0.64 10 (9-10)	8.38 ± 1.5 9 (8-9)	0.000
Visfatin (ng/ml)	11.04 ± 7.14 13.52 (3.57-17.97)	8.88 ± 7.91 6.69 (1.37-17.85)	0.616

Table 1
COMPARISON BETWEEN PREECLAMPSIA AND CONTROL PATIENTS IN TERMS OF GESTATION DURATION, BIRTH WEIGHT OF CHILDREN, AND VISFATIN INFLUENCE ON GESTATION AGE AND APGAR SCORE

Variable	Preeclampsia		p-value
	Medie	Severa	
Week of pregnancy	36.45 ± 3.54 38 (34-39)	36.2 ± 2.39 36 (34-38)	0.363
Systolic TA (mmHg)	147.45 ± 6.4 150 (140-150)	169.5 ± 7.62 170 (170-171.25)	0.000
Diastolic TA (mmg)	96.83 ± 5.22 100 (90-100)	106.5 ± 8.84 110 (100-111.25)	0.002
Gestation duration			0.542
Trim I	12 (40%)	6 (60%)	
Trim II	14 (46.7%)	3 (30%)	
Trim III	4 (13.3%)	1 (10%)	
Number of previous births	1.34 ± 0.55 1 (1-2)	1.1 ± 0.32 1 (1-1)	0.346
Newborn weight (g)	2493.1 ± 692.33 2700 (1835-3000)	2202 ± 485.38 2300 (1800-2630)	0.089
Apgar index	8.66 ± 1.59 9 (8-9.5)	7.6 ± 0.84 8 (7.5-8)	0.000
Visfatin (ng/ml)	9.91 ± 8.25 11.03 (1.22-18.07)	5.89 ± 6.23 3.23 (1.49-9.7)	0.437

Table 2
COMPARISON BETWEEN PATIENTS
WITH MODERATE AND SEVERE
PREECLAMPSIA

Table 3
VISFATIN INFLUENCE ON THE BIRTH WEIGHT OF FOETUSES

Model	Unstandardized Coefficients		Standardized Coefficients		Sig. = p-value	Collinearity Statistics	
	B	Std. Error	Beta	t		Tolerance	VIF
(Constant)	7.260	.310		23.399	.000		
visfatin	.028	.030	.130	.949	.347	.986	1.014

blood pressure (p-value = 0.000), weight of the newborn (p-value = 0.000), the Apgar score. Descriptive parameters show that the pre-eclampsia group gave birth much earlier than the control group and the blood pressure (BP) was much higher (SBP and DBP). As expected, significant differences exist in terms of the weight at birth of the baby, pre-eclamptic mothers had babies with much lower weight and poorer performance at birth (measured through the Apgar index). In respect to the hormones measured, the patients group had values lower than the control one for the Visfatin, but differences did not turn out to be significant.

The same variables present significant differences between groups when the sample is divided into three parts - control group, medium pre-eclampsia and severe pre-eclampsia. As expected, the latter group had the lowest birth age, the highest TA, the lowest Apgar scores and birth weight. For Visfatin, no statistical differences were found between the groups analyzed (p-value = 0.529 >> 0.05).

Lastly, in table 2, we compared only the pre-eclampsia group, to see if any statistically significant differences are to be found. Results remain the same for TA and the Apgar score. The group that had much higher TA had significantly lower Apgar scores at birth. The weight of the newborn loses its significance, the two groups having approximately the same features for this variable. For Visfatin, results show that this hormone does not influence the occurrence of preeclampsia.

The table 3 shows that Visfatin does not influence birth weight. The pleiotropic effects of Visfatin are expressed in a series of physiological and pathophysiological processes, changes in its homeostasis being related to obesity, type II diabetes, sepsis, acute pneumopathy and various gestational complications, especially preeclampsia. The role of Visfatin in pregnancy is still difficult to quantify given the discordance existing among the results of the various studies. What is known precisely is that it intervenes in foetal, placental metabolism, both in part and combined.

Morgan et.al suggested as early as 2008 that Visfatin is involved in placentation and its proper functioning [10]. In 2011, Kim et al. demonstrated that H3-K9 / K14 histone acetylation plays a role in regulating the expression of the Visfatin gene in mice during placentation [11].

In preeclamptic patients, Visfatin serum levels are lower than in those without preeclampsia, with no statistically significant differences. The lowest serum levels are found in the most severe forms. The absence of statistically significant differences can be explained by the existence of a multitude of processes that regulate the secretion of Visfatin, among which we mention: birth, uterine distension, intraamniotic infections, low birth weight from mechanisms other than preeclampsia, preterm delivery, gestational diabetes [5].

Our study is consistent with that of Hu et al. [12], which showed a decrease in Visfatin expression in preeclampsia patients. The proposed explanation is related to insulin homeostasis, as Visfatin has insulinomimetic properties. Thus, a low visfatin level causes a decrease in insulin sensitivity, and pregnancy is known to be a condition in which this metabolic change occurs in extremis, resulting in gestational diabetes. Another proposed explanation is related to the down-regulation of Visfatin in the adipose tissue, a process in which other adipokines certainly intervene, including the family of interleukins 6 [12]. Data related to Visfatin serum levels are contradictory. Unlike the study of Hu et al. [12], Romao et al have suggested that overexpression of EMMPRIN (extracellular matrix metalloproteinase inducer) and hyaluronan would result in augmentation of the inflammatory response, elevated Visfatin levels and tissue damage, observed in preeclampsia patients [13]. This study also failed to show significant differences in Visfatin serum levels in patients with preeclampsia compared to healthy patients [13]. Our study did not demonstrate a clear link between Visfatin and the occurrence of preeclampsia, suggesting that this hormone does not trigger the process, more likely it

intervenes in regulating negative effects. This was also stated by authors who have found low levels of Visfatin in preeclampsia patients coinciding with higher levels in the blood of newborns with low birth weight, which can be explained by an increased transport from the maternal blood into the foetal blood [12, 14]. Although some authors found elevated Visfatin serum concentrations in newborns with low birth weight compared to those with normal weight, other published no statistically significant differences, and, according to them, foetal circulation cannot be the source of Visfatin during the gestational period [15]. The diversity of results could be explained by the fact that the samples are described differently, the population included is different in terms of ethnicity, gestational age and body mass index (very difficult to calculate in pregnant patients). In the literature there is evidence of Visfatin levels in overweight people. Fukuhara et al. showed in 2005 that the plasma level of Visfatin correlates with the amount of perivisceral fat detected by CT in human subjects. He also showed, but on toy models, that serum Visfatin increases as they become obese [1]. A meta-analysis of the studies conducted so far into its role in metabolism, has shown that circulating Visfatin is correlated with obesity and overweight [2]. In addition, the presence of undetected subclinical infections [16] may alter the inflammatory profile of the subjects included in the study.

Conclusions

In this study we found lower Visfatin serum levels in preeclamptic patients versus control patients, but these differences did not prove to be statistically significant. The lowest serum levels were recorded in patients with the most severe form of preeclampsia. Plasma concentration of Visfatin was not significantly different in patients with preeclampsia who gave birth to normal weight foetuses, compared to those who gave birth to low-weight foetuses.

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Manuscript received: 15.03.2017