Abstract. – Expedited research on Obesity has confirmed that, adipose tissue is highly active in secreting a variety of proteins, one among them is visfatin. It was originally identified as Pre B cell Colony Enhancing Factor (PBEF), to be secreted by the lymphocytes and can act as a cytokine with immune regulatory action. Besides, it acts as Nicotinamide phosphoribosyl transferase (Nampt), an enzyme involved in the NAD+ salvage pathway. It has been shown to help in the regulation of glucose homeostasis, but whether it binds to insulin receptor and exerts insulin mimetic activity is still a controversy. Visfatin has antiapoptotic activity and has a regulatory role in inflammation. Several studies have identified changes in the circulatory levels of visfatin in diseases. Notable among them are obesity, diabetes mellitus, kidney diseases and bone disorders. It is a molecule of clinical relevance and could be a promising biomarker with diagnostic and prognostic significance.

Key Words: Adipokines, Visfatin, PBEF, Nampt.

Introduction

White adipose tissue is no longer just an inert organ or organ which stores excess of energy in form of triglycerides. Research in the field of nutrition or non communicable diseases like obesity have been extensively covered, depicting adipose tissue to be actively secreting many adipokines viz leptin, adiponectin, resistin, visfatin and adipocytokines like TNF-α, MCP-1, PAI, IL-6, complement factors etc. These substances are released not just by adipocytes, but also by the connective tissue matrix and immune cells of obese white adipose tissue. This shows that adipose tissue is complex, essential and highly active metabolic endocrine organ. Much is known about leptin, adiponectin, resistin etc but very little is known about visfatin as it is discovered recently and also has been found to be ubiquitously expressed and is associated with variety of functions in different cell types. Keeping this in view the aim of this article is to summarise in brief about visfatin.

History

Visfatin was identified first as Pre B cell Colony Enhancing Factor (PBEF), to be secreted by human peripheral blood lymphocytes. It acts
like an enzyme (Nicotinamide phosphoribosyl transferase) Nampt, which is involved in NAD⁺ salvage pathway. Recently, PBEF was identified by Fukuhara et al.⁵ as visfatin a novel adipokine - a protein mediator secreted by fat cells (high levels of expression in visceral fat cells). Analysis of the amino acid sequence of visfatin revealed it to be identical with PBEF/Nampt.

Three different biological names with three different functions for a single protein (as amino acid sequence is same for these three proteins) have made it, unique and biologically indispensable; hence the names are used interchangeably.

Structure of Visfatin

Visfatin is a 52-kDa protein,¹ is active as a dimer, with each monomer containing 491 amino acids in humans. This has been proved by the fact that visfatin/PBEF/Nampt has two active sites at the interface of the dimeric protein, suggesting that dimerization is essential for the catalytic activity of the enzyme⁴. Each monomer contains 19 β-strands and 13 α-helices and is organized into two structural domains⁵. However, the protein lacks signal peptide, so whether it is released from a viable cell or dead cell is still to be answered. In humans the gene is located on the long arm of chromosome 7 between 7q22.1 and 7q31.33⁶. Visfatin gene is well preserved during evolution. For example, the canine visfatin protein sequence is 96% and 94% identical to human and rodent visfatin, respectively⁷.

Distribution in Organs and Organelles

Bone marrow, liver and muscle have been reported to be the tissues with the highest expression levels of this protein¹, followed by brain, kidney, spleen, testis, lung, but preferentially expressed in visceral fat than in subcutaneous fat and upregulated in some animal models of obesity. It is also released by fetal membranes during pregnancy⁸. This hormone is found in the cytoplasm as well as the nucleus of cells⁷.

Functions

PBEF as a Cytokine and Immunomodulator

Visfatin can be considered a new proinflammatory adipocytokine. It dose-dependently up-regulates the production of the pro- and anti-inflammatory cytokines IL-1β, IL-1Ra, IL-6, IL-10, and TNF-α in human monocytes. These cytokines play a substantial role in a wide range of infectious and inflammatory diseases⁹.

High circulating visfatin levels have been observed in rheumatoid arthritis¹⁰ and acute lung injury¹¹. Significantly higher visfatin mRNA expression was found in inflamed Inflammatory Bowel Disease; colonic biopsies suggests that the colonic mucosa is a potential source of elevated visfatin plasma levels. By histological examination, it has been identified that potential cellular sources of visfatin in inflamed colonic tissue included APCs (acute phase cells), like dendritic cells and macrophages, as well as epithelial cells⁵. There are several reports demonstrating enhanced tissue expression of visfatin in inflammatory conditions including clinical sepsis⁶, and severe generalized psoriasis¹². Macrophages have been suggested as a significant source of this protein in addition to adipose cells, as visfatin/PBEF/Nampt-positive macrophages have been identified in adipose tissue and in the submucosa of the colonic wall⁹.

PBEF/Nampt/Visfatin as an Enzyme

It is involved in the salvage pathway of NAD⁺. NAD⁺ synthesis in mammals occurs by one of two principle pathways. It can be synthesized from the de novo pathway or from one of the three salvage pathways. De novo synthesis begins with tryptophan, which undergoes several reactions to form quinolinic acid, which is converted to nicotinic acid mononucleotide (NaMN) by quinolate phosphoribosyltransferase (Qprt). NaMN is then adenylylated by NaMN adenylyltransferase (Nmnat) to form nicotinate adenine dinucleotide (NaAD), which is converted to NAD⁺ by glutamine-dependent NAD⁺ synthetase. The three salvage pathways are a) nicotinic acid pathway, nicotinic acid salvaged by NAPRTase (Npt) to form NaMN; b) nicotinamide pathway, nicotinamide is salvaged by Nampt/PBEF to MNM, which is adenylylated to form NAD⁺ by Nmnat; c) nicotinamide ribose pathway, nicotinamide ribose is salvaged by nicotinamide riboside kinases (Nrk) to form NaMN¹³. Our interest of salvage pathway is the one where visfatin/Nampt acts like an enzyme helping in the production of NAD⁺.

Why so much of importance to NAD⁺? NAD⁺ is an essential cofactor in a number of fundamental intracellular processes like (a) Transfer of electrons during redox reactions; (b) To modulate
the activity of key regulators of cellular longevity; (c) To serve as a substrate for the generation of other biologically important molecules. Much is known about its redox activity, but other activities are still in initial stages of research. NAD+ is increasingly recognized to be involved in the regulation of intracellular signaling. NAD+ is an essential cofactor for the activity of a family of Class 3- NAD+-dependent HDACs (histone deacetylases) known as SIRTs (silent information regulator 2). It binds to NAD+ and a protein (target protein) that contains an acetylated lysine. It catalyzes the formation of acetylated ADP-ribose by deacetylation of the lysine residue of the target protein. These sirtuins (Sir2) and its human orthologs consume NAD+ and generate increased production of nicotinamide and a novel metabolite O-acetyl ADP-ribose, as they hydrolytically remove an acetyl group from a lysine residue of their target proteins. However, recently it has been found that the intracellular levels of NAD+ (increased) and nicotinamide (decreased) are very important for certain cell survival reactions, including those linked to the sirtuin family of protein deacetylases. To replenish the decreased stores of NAD+ the salvage pathways are must, specially the nicotinamide pathway, which involves the Nampt enzyme. Sirtuins have been implicated in influencing aging and regulating transcription, apoptosis and stress resistance. So, we can say that Nampt indirectly helps in the longevity of the cells life span. This was proved by the fact that Nampt extends the lifespan and promotes the maturation of human SMCs (smooth muscle cells) by activating SIRT1.

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**Insulin Like Function?**

Insulin once secreted from B-cells of pancreas into circulation, binds to the insulin receptors of insulin sensitive targets, leading to internal signalling etc. These things are well known fact. Hence when we say insulin like activity of visfatin it means to say it has its journey in showing its function same as that of insulin. The binding affinity of visfatin/PBEF/Nampt to the IR (insulin receptor) was found to be similar compared with that of insulin. Many studies have demonstrated an increased levels of visfatin in diabetes mellitus. However, in an experiment conducted on cohort of obese patients did not show any correlation between PBEF/visfatin to glucose infusion. Whether visfatin binds to the IR remains controversial. This made Fukuhara et al to retract their article. But they still stand up to their conclusions.

Whether visfatin binds to insulin receptors and exerts its insulin mimetic activity is still a controversy, but recent research has shown that Nampt/visfatin-mediated systemic NAD+ biosynthesis is necessary for β cell function, suggesting that visfatin helps in regulation of glucose homeostasis.

**Visfatin as an Anti Apoptotic Molecule**

Apoptosis is a programmed cell death. It is a phenomenon of widespread biological importance. Apoptosis of inflammatory cells is important for the resolution of inflammation. It is necessary for tissue kinetics and leads to removal of unwanted cells without causing tissue injury. Removal of intact neutrophils is necessary to prevent chronicity of the disease, because it leads to recognition of intact senescent neutrophils that have not necessarily disgorged their granule contents. These processes may represent a mechanism for the removal of neutrophils during inflammation that also serves to limit the degree of tissue injury. Circulating neutrophils from patients with SIRS (systemic inflammatory response syndrome) or from patients who have undergone major elective surgery show delayed expression of constitutive programmed cell death, and antiapoptotic factors were present in their general circulation. While prolonged neutrophil survival may represent an appropriate adaptive response to injury, the presence of activated and apoptosis-resistant cells in an anti-apoptotic environment may contribute to the systemic inflammatory injury characteristic of SIRS and predispose to the development of the multiple organ dysfunction syndromes. PBEF plays a requisite role in this inhibition that is inhibition of apoptosis.

Transcription of the PBEF gene is increased in neutrophils from septic patients; prevention of PBEF translation through the use of an antisense oligonucleotide largely restores the normal kinetics of apoptosis. Moreover, the incubation of quiescent neutrophils from healthy volunteers with recombinant PBEF results in dose-dependent inhibition of apoptosis, and antisense PBEF prevents the inhibition of apoptosis that results from exposure to LPS (Lipopolysaccarides) or to a variety of host-derived inflammatory cytokines. The mechanism of PBEF-mediated inhibition of apoptosis is unclear.
Visfatin and Related Studies

Visfatin and Obesity
Circulating visfatin levels are closely correlated with VAT (White Adipose Tissue) accumulation, visfatin mRNA levels increase in the course of adipocyte differentiation, and visfatin synthesis is regulated by several factors, including glucocorticoids, TNF-α, IL 6, and GH. Visfatin plasma concentrations and visceral visfatin mRNA expression correlated with measures of obesity but not with visceral fat mass or waist-to-hip ratio. In addition, differences in visfatin mRNA expression between visceral and subcutaneous adipose tissue in humans, was also not significant. Visfatin levels have been shown to be increased in children of more BMI indicating important implication of this new adipokine in inflammatory mechanisms of obesity starting already in childhood. Visfatin were shown to be increased in females with obesity (visceral obesity). Decrease in circulating visfatin was found in morbidly obese women who lost more than 20% of their BMI, also increased plasma visfatin concentrations in morbidly obese subjects are reduced after gastric banding. These studies show that more the BMI (obesity) more the visfatin levels and levels decrease after weight loss.

Visfatin and Diabetes
Circulating visfatin is increased with progressive beta-cell deterioration. Exercise training lowers plasma visfatin concentrations in patients with type 1 diabetes mellitus.

Visfatin and Kidney Disorders
Loss of renal function is accompanied by increased circulating active visfatin, as it is released from the damaged endothelial cells.

Visfatin, Bone Metabolism and Disorders
Visfatin/PBEF/Nampt exerts its effects on bone metabolism by acting on human osteoblasts, by increasing glucose uptake, stimulating expression of osteogenic markers at the mRNA and protein levels, and also causing an increase in mineralization of osteoblasts in a manner similar to insulin. Chronic inflammation affects bone metabolism and is commonly associated with the presence of osteoporosis. Bone loss is directed by various immune mediators like visfatin/PBEF/Nampt suppressed osteoclastogenesis and inhibited the differentiation of osteoclast precursors.

Visfatin and CSF
Visfatin concentrations in human CSF decrease with rising body fat, supporting the assumption that visfatin transport across the blood-brain barrier is impaired in obesity and that central nervous visfatin insufficiency or resistance are linked to pathogenetic mechanisms of obesity.

Estimation of Visfatin
Estimation of visfatin in serum is done by ELISA. No difference in the levels of visfatin was observed between males and females. The normal range being 15.8 ± 16.7 ng/ml.

Conclusion
Visfatin also known as PBEF/Nampt is a newer adipocytokine with diverse regulatory and metabolic roles. It is expressed in tissues like bone marrow, liver, muscles, brain, kidney, spleen, testis, lungs, fetal membranes but preferentially expressed in visceral adipose tissue and is known to be upregulated in obesity (in animal models). It acts as an enzyme in NAD+ salvage pathway. It has proinflammatory and anti apoptotic potentials and play important role in infectious and inflammatory diseases.

Circulating visfatin levels have been shown to be influenced by conditions like obesity, diabetes mellitus, kidney disease, bone disorders and there is scope for future research to establish its diagnostic/ prognostic values. Also no confirmatory findings are there regarding its increase or decrease in fed and fasting state, so tough to say whether it has insulin like activity. Other functions do not show much of its hormone like activity, so we doubt whether it is a classical adipocytokine or not!

References
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