Is eNAMPT/visfatin a potential serum marker of papillary thyroid cancer?

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Abstract

Purpose: The role of nicotinamide phosphoribosyltransferase (NAMPT)/visfatin in a more aggressive course of many malignancies has been proven. Previous studies have noticed the importance of visfatin in thyroid neoplastic tissue, but the diagnostic and prognostic value of its serum concentration has not been investigated so far. Our study aimed to consider whether extracellular NAMPT (eNAMPT) could be a potential serum marker in recurrent papillary thyroid cancer (PTC).

Methods: It was a prospective observational study with consecutive enrolment. We recruited 100 patients with PTC after thyroidectomy with postoperative ^{131}I ablation and 100 healthy controls. Also, 50 randomly selected patients underwent laboratory assessment (including eNAMPT serum concentration by ELISA Assay Kit, TSH, free thyroid hormones, TSH-stimulated thyroglobulin Tg, antibodies – TgAbs, TPOAb) and body composition analysis twice: at admission and 6 months after being on suppressive levothyroxine doses. TSH-stimulated Tg of 1 ng/ml was defined as the cutoff value for predicting disease status as complete remission ($n = 55$) and recurrent or persistent structural disease ($n = 45$).

Results: The visfatin serum concentrations in patients diagnosed with PTC and in healthy subjects were not statistically significantly different ($p = 0.9425$). The eNAMPT levels were also similar in disease-free patients and the ones with tumour relapse. Besides, ROC curve analysis did not detect eNAMPT as a biomarker of PTC.

Conclusion: We have not found visfatin as a potential serum marker of papillary thyroid cancer. Also, eNAMPT has no prognostic value in assessing the risk of disease recurrence or metastasis in PTC management.

Keywords: nicotinamide phosphoribosyltransferase, papillary thyroid cancer, recurrence, serum marker, visfatin

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Introduction

Nicotinamide phosphoribosyltransferase (NAMPT) catalyzes the rate-limiting step of nicotinamide adenine dinucleotide (NAD+) synthesis.[1] NAMPT is also called visfatin or pre-B-cell-enhancing factor. NAMPT/visfatin was first described in 1994 by Samal *et al.*[2] as an active protein in extracellular space, secreted by pre-B cells. In 2005, Fukuhara *et al.*[3] identified it as an adipokine secreted by visceral adipose tissue in obese patients and called visfatin. Now, it is clear that NAMPT is produced by pre-B cells and adipocytes and is detectable in most cell types, for example, macrophages, leucocytes, primary glial cells or fibroblasts.[4] It regulates various signalling pathway components, including AMP-activated protein kinase (AMPK), PI3K/Akt and ERK1/2.[5] Therefore, NAMPT is essential for energy homeostasis – downregulated, it promotes apoptosis in cancer cells and attenuates tumour growth. Inversely, NAMPT expression is upregulated in many different human malignancies, where it

promotes cell growth and survival, DNA synthesis, mitochondrial biogenesis and angiogenesis.[6–10] The angiogenic effects of NAMPT are mediated by activation of ERK1/2 in endothelial cells. NAMPT can also activate the signal transducer and activator of transcription 3 (STAT3) protein and increase tyrosine phosphorylation, nuclear translocation and DNA-binding activity in human endothelial cells. This process, in turn, up-regulates endothelial IL-6 *mRNA* and protein levels.[11] Higher NAMPT expression correlates with a more aggressive clinical course in some malignancies.[6,7,11–15] The reason for this might be stress conditions, including hypoxia and oxidative stress as features of the tumour environment.[4]

Extracellular NAMPT (eNAMPT) circulates in the plasma/serum, and intracellular (iNAMPT) can be found in the cytoplasm, mitochondria and nucleus in the majority of cells. eNAMPT is probably released in plasma by cell lysis, cell death or through a nonclassical pathway. Abnormalities in circulating eNAMPT levels are connected with the pathogenesis of several human diseases. NAMPT plasma concentration is elevated in cancer patients and inflammatory disorders and related to the inflammation grade. eNAMPT stimulates the production of many proinflammatory cytokines and enhances the production of IL-1α, IL-1β, IL-6, IL-8 and tumour necrosis factor α (TNF-α). According to the recent research serum, eNAMPT was reported as a useful biomarker of malignant potential, stage progression and prognosis, which can help monitor the disease. Moreover, it is associated with cancer therapy resistance. However, not all studies have determined elevated plasma eNAMPT concentrations in cancer patients than their controls.[16]

We found *NAMPT mRNA* expression significantly higher in thyroid cancers, and it was correlated with tumour stage.[7] In immunohistochemistry, NAMPT overexpression was reported in differentiated thyroid cancers.[1] We also reported increased serum visfatin concentration and its tissue and leukocyte overexpression in autoimmune thyroid diseases, which might speak for a link between chronic inflammation and malignancy.[17–20]

So far, no investigations have been carried out to define the relevance of serum visfatin in managing patients with differentiated thyroid cancer. This is the first study to investigate the potential feasibility of a blood-based diagnostic visfatin serum test as a marker in patients with papillary thyroid cancers based on overexpressed *mRNA* and protein in thyroid cancer *versus* benign pathology.

Patients and methods

All methods were carried out in accordance with relevant guidelines and regulations.

Study design and patient enrolment

It was a prospective observational study with consecutive enrolment (Figure 1). We included patients thyroidectomized for papillary thyroid cancer after radioiodine ablation, who had withdrawn levothyroxine for 4 weeks before admission. All patients underwent total or near-total thyroidectomy with postoperative ^{131}I ablation of residual thyroid tissue. Randomly selected patients underwent body composition analysis and were assessed twice: at admission and 6 months after being on suppressive levothyroxine doses. Exclusion criteria were acute or chronic diseases (autoimmune diseases including elevation of antithyroid antibodies or features of autoimmune thyroiditis in a histopathological examination, infection and any other cancers), medications taken regularly (potentially affecting body composition or visfatin concentrations). Tumour staging was carried out according to the 8th AJCC TNM staging system.[21,22] All patients underwent scanning under endogenous thyroid-stimulating hormone (TSH) stimulation with diagnostic activities of ^{131}I to confirm the presence and extent of residual thyroid tissue or distant metastases.

Moreover, every patient underwent an ultrasound of the neck with cervical lymph nodes assessment. The sex- and age-matched control group was recruited. Serum concentrations of eNAMPT/visfatin, TSH, FT4, FT3, thyroglobulin (Tg), antithyroperoxidase antibodies (TPOAbs), antithyroglobulin antibodies (TgAb), fasting glucose and insulin were measured in the study and control groups. The local ethical committee approved the study, and all patients signed informed consent.

Patients' outcome definition

Patients' disease status was defined as complete remission or persistent/recurrent structural disease according to clinical evaluation (including thyroglobulin measurement, diagnostic ^{131}I-WBS, chest

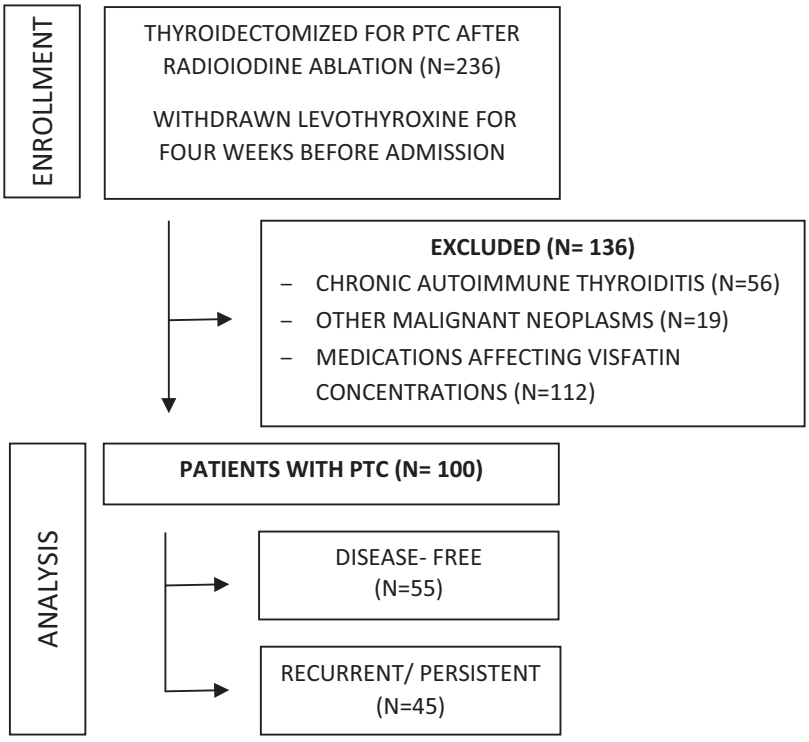

Figure 1. The study flow chart.

X-ray, neck ultrasonography, followed by computed tomography (CT), magnetic resonance imaging (MRI) or positron emission tomography (PET)/CT scans in the case of abnormal initial examination). TSH-stimulated Tg of 1 ng/ml was defined as the cutoff value for predicting disease status.

Laboratory measurements

ELISA Assay Kit from Phoenix Pharmaceuticals was used to assess visfatin levels. Electrochemiluminescence technique was applied for estimation of TSH, FT4 and FT3 concentrations (normal ranges: TSH 0.27–4.2 mU/l; FT4 11.5–21.0 pmol/l; FT3 3.9–6.7 pmol/l). TSH-stimulated thyroglobulin (Tg) was measured with immunoradiometric assay (Brahms Diagnostica Berlin, Germany), and interfering antibodies against thyroglobulin (TgAbs) were measured with radioimmunoassay (Brahms Diagnostica Berlin, Germany). TPOAb was assessed by radioimmunoassay (normal ranges: <34 IU/ml). Glucose levels were estimated using Hitachi Cobas e601 chemiluminescent analyzer (Roche Diagnostics), and insulin concentration was assessed using an ELISA kit from Phoenix Pharmaceuticals.[17,20,23]

Body composition analysis

Body composition measurements were performed with the total body bioimpedance analyzer Tanita MC 180 MA II (Tanita, Japan) in randomly selected patients at admission and 6 months after on suppressive doses of levothyroxine.[23]

Statistical analysis

Statistical analysis was performed with MedCalc Statistical Software version 19.1.5 (MedCalc Software bv, Ostend, Belgium; https://www.medcalc.org; 2020). Normality was analysed by the D'Agostino–Pearson test. Comparison of results between the study and control groups was performed with the Mann–Whitney test. When data did not follow a normal distribution, a comparison of the analysed parameters within the subgroup at admission and 6 months after on suppressive doses of levothyroxine was performed using the nonparametric Wilcoxon test for paired data. Paired samples *t* test was used to compare analysed parameters in normally distributed data. Spearman's rank correlation coefficient was used to find relationships between analysed parameters. Receiver operating characteristics (ROC) curves

Table 1. Clinical data of patients.

Characteristic	Disease-free *N* = 55	Persistent/ recurrent structural disease *N* = 45	Healthy controls *N* = 100
Age (years), Me (IQR)	41 (32–53)	50 (34–60.75)	45 (33–57)
Sex	F 51 (92.7%) M 4 (7.3%)	F 36 (80%) M 9 (36%)	F 87 (87%) M 13 (13%)
Weight (kg), Me (IQR)	70.3 (62.8–82.8)	70.9 (59–86)	70.6 (60–83.2)
BMI (kg/m^2)	25.6 (22.3–29.4)	25.6 (21.8–29.8)	25.8 (22.3–29.6)
Staging at diagnosis	I 26 II 8 III 16 IV 5	I 9 II 4 III 22 IV 10	–
Lymph node involvement at diagnosis	8	23 –	–
Distant metastases at diagnosis	0 2 (lungs)		
Follow-up (months)	45 (34–67)	54 (36–87)	
^{131}I cumulative activity (mCi)	90 (60–104)	270 (141–477)	–
^{131}I WBS positive (number of patients)	0	36 (lymph nodes in 34 pts; bones in 2 pts; lungs in 4 pts)	–

N, number; Me, median; F, female; M, male; BMI, body mass index; WBS, whole body scan.

were calculated to determine serum eNAMPT/visfatin concentration's potential to discriminate between patients with thyroid cancers and healthy controls. An optimal cutoff point was calculated according to the highest accuracy (minimal false-negative and false-positive results). The area under the ROC curve (AUC) was used to check the particular parameter's prognostic value. To achieve 80% power at two-sided 5% significance level and the AUC of 0.725, the required sample size of 48 participants (24 patients with thyroid cancer and 24 healthy controls) was calculated. A *p*-value of less than 0.05 was considered statistically significant.[17,20,23]

Results

Clinical and biochemical evaluation

We recruited 100 patients with papillary thyroid cancer (PTC) and 100 sex- and age-matched healthy controls. Also, 50 randomly selected patients underwent laboratory assessment and body composition analysis at admission and 6 months after being on suppressive levothyroxine doses. According to clinical assessment, 55 patients (51 women and 4 men) were classified as disease-free, and 45 (36 women and 9 men) with persistent/recurrent structural disease. The characteristic of patients is provided in Table 1.

Changes of biochemical parameters and body composition after LT4 withdrawal and under LT4-suppression

Table 2 shows changes in biochemical parameters in 50 randomly selected patients 6 months after implementing suppressive doses of LT4. TSH level decreased, while FT4 and FT3 levels increased significantly ($p < 0.0001$). Visfatin serum levels decreased.

The body composition changes in patients with thyroid cancers at admission and 6 months after on suppressive doses of levothyroxine are presented in Table 3. We observed decrease in

body weight (median decrease 3.7 kg/6 months), body mass index (BMI; median decrease 0.9 kg/m^2/6 months), fat content (median decrease 1.5%/6 months), fat mass (median decrease 2.9 kg/6 months), free fat mass (median decrease 1.2 kg/ 6 months), muscle mass (median decrease 1.4 kg/6 months) and visceral fat index (median decrease 1/ 6 months).

Efficacy of serum visfatin in identifying patients with papillary thyroid cancer

Comparisons of visfatin serum levels between patients with papillary thyroid cancer and healthy controls revealed similar concentrations in both groups ($p = 0.9425$). Also, the ROC curve analysis did not find visfatin as a biomarker of papillary thyroid cancer.

Efficacy of serum Tg, TgAbs and visfatin in identifying patients with persistent/recurrent structural disease

The ROC curve analysis showed that the cutoff point of TSH-stimulated Tg higher than 0.94 ng/ml was the best predictor of cancer recurrence (sensitivity = 84.44%; specificity = 90.91%; AUC = 0.925; $p < 0.001$). TgAbs and visfatin did not reflect disease status (Table 4). Visfatin serum levels were similar in disease-free patients with papillary thyroid cancer and patients with tumour relapse (Table 4).

Discussion

Our study neither showed differences in visfatin serum concentrations between healthy controls and patients with papillary thyroid cancers, nor between recurrent and nonrecurrent patients. Otherwise, our analysis confirmed that the TSH-stimulated thyroglobulin (Tg) higher than 0.94 ng/ml was the best predictor of cancer recurrence with relatively high sensitivity and specificity. The body composition changes in patients with thyroid cancers at admission and 6 months after on suppressive doses of levothyroxine (LT4) were statistically significant. We have observed a decrease in eNAMPT/visfatin concentrations in iatrogenic subclinical hyperthyroidism achieved 6 months after implementation of LT4-suppressive therapy.

So far, to our best knowledge, this is the first investigation carried out to evaluate the diagnostic and prognostic relevance of serum visfatin levels in the management of patients with differentiated thyroid cancer. The main limitation of this study is its cross-sectional design. Our findings must be interpreted according to the study

Table 2. Changes of biochemical parameters in randomly selected patients with thyroid cancers at admission and six months after on suppressive doses of levothyroxine.

Median (25–75%)	Before	After	*p*
TSH (μIU/mL)	100 (67.2–100)	0.1 (0.03–0.23)	<0.0001
FT4 (pmol/L)	2.7 (1.8–4.0)	20.0 (19.6–21.2)	<0.0001
FT3 (pmol/L)	1.3 (1–2.7)	4.9 (4.2–5.7)	<0.0001
Visfatin (ng/ml)	7.75 (6.9–8.8)	7.45 (6.8–8.6)	0.0001

TSH, thyroid-stimulating hormone; FT4, free thyroxine; FT3, free triiodothyronine.

Table 3. Changes of body composition in randomly selected patients with thyroid cancers at admission and 6 months after on suppressive doses of levothyroxine.

Median (25–75%)	Before	After	*p*
Body weight (kg)	68.4 (58.9–74.7)	64.7 (55.4–70.1)	<0.0001
BMI (kg/m^2)	24.6 (21.3–29.5)	23.7 (19.9–28.6)	<0.0001
FC (%)	27.9 ± 6.6	26.4 ± 6.8	<0.0001
FM (kg)	19.5 (15.3–23.7)	16.6 (13.8–21.4)	<0.0001
FFM (kg)	47.1 (43.6–52.9)	45.9 (42.7–50.6)	<0.0001
MM (kg)	45.9 ± 6.9	44.5 ± 7.5	<0.0001
VFI	5 (3-6)	4 (2-6)	<0.0001

BMI, body mass index; FC, fat content; FM, fat mass; FFM, free fat mass; MM, muscle mass; VFI, visceral fat index.

Table 4. Efficacy of serum Tg, TgAbs and visfatin in identifying patients with persistent/recurrent structural disease.

Assay	Sensitivity (%)	Specificity (%)	*p*
Serum Tg	84.4	90.9	<0.0001
Serum TgAbs	64.44	50.91	0.6142
Serum visfatin	53.3	60.0	0.9290

PTC, papillary thyroid cancer; Tg, thyroglobulin; TgAbs, antithyroglobulin antibodies.

design. In case control studies, there is a potential selection bias, especially in the control group. This may lead to masking the connection of serum visfatin and PTC. Controls were selected randomly to reduce bias. We applied strict inclusion and exclusion criteria, what allowed to avoid factors, which might potentially blind the results. In addition, our strength is the sample size which is sufficient to give statistically significant results.

Despite evidence in the literature for upregulation of visfatin/NAMPT in thyroid cancer tissue, the same is not valid for serum.[1,7] Serum markers need a considerable difference in concentration to be useful for diagnostic purposes. In contrast, identifying markers in tumour samples is much easier due to comparing cancer and adjacent healthy tissue.

The importance of thyroid hormones and their role in controlling body mass and composition is well known.[23–26] TSH suppressive LT4 therapy is an established component of the management of thyroid carcinoma growth in patients with differentiated thyroid carcinoma. In our study, suppressive doses of levothyroxine caused a decrease in body weight resulting from a decrease in fat and muscle masses. It might be explained by the increased rest energy expenditure in patients on suppressive doses of levothyroxine.[27] However, some studies analysing a smaller number of patients did not report a decrease in body weight or fat content in athyreotic patients in an iatrogenic subclinical hyperthyroid phase when compared with the hypothyroid state achieved after levothyroxine withdrawal.[28,29]

In our recent study, lower eNAMPT/visfatin concentrations in the hyperthyroid state were observed compared with the euthyroid phase in females with Graves' disease.[23] As mentioned earlier, the decrease in eNAMPT/visfatin serum concentrations might be related to the decrease in fat tissue.

Further studies are needed to investigate the potential role of serum visfatin in diagnosis of medullary thyroid cancers.

Conclusion

In conclusion, eNAMPT/visfatin is not elevated in the serum of patients with PTC. Also, our results point that visfatin is not upregulated in serum of patients with persistent/recurrent structural disease; therefore, it is not a potential serum marker of the disease. Levothyroxine withdrawal and suppressive therapy impact body composition and eNAMPT/visfatin serum levels, so patients' dietary and lifestyle education seem to be an essential part of a lifelong therapy in those patients.

Author contributions

Nadia Sawicka-Gutaj: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Resources; Writing – original draft.

Paulina Ziółkowska: Data curation; Writing – original draft.

Aleksandra Derwich: Data curation; Resources; Writing – original draft.

Paweł Gut: Data curation; Supervision; Writing – review & editing.

Agata Czarnywojtek: Conceptualization; Data curation; Supervision; Validation; Writing – review & editing.

Michał Kloska: Conceptualization; Formal analysis; Investigation; Methodology; Writing – review & editing.

Marek Ruchała: Supervision; Writing – review & editing.

Conflict of interest statement

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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Ethics approval and consent to participate

The Ethical Committee of Poznan University of Medical Sciences approved the present study (Reference: nr 351/14). Written informed consent was obtained from all patients.

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Availability of data and materials

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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