Anti-vimentin antibodies and neuron-specific enolase in children with neurofibromatosis type-1

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Abstract

OBJECTIVES: The aim of the study was to investigate the relationship of serum levels of neuron-specific enolase, anti-vimentin IgG, and anti-vimentin IgM antibodies in patients with neurofibromatosis type 1 and associated tumors (optic glioma, and plexiform neurofibroma).

METHODS: Measurement of neuron-specific enolase and anti-vimentin antibodies were performed in 131 children and adolescents (67 males, mean age 10 years, range 4–19 years; 64 females, mean age 11 years, range 1–20 years) with three different forms of neurofibromatosis type 1 and in control group of 40 individuals (20 males, mean age 9 years, range 1–19 years and 20 females, mean age 12 years, range 3–18 years).

RESULTS: Anti-vimentin IgG, IgM antibodies and NSE showed similar ability to distinguish between neurofibromatosis type 1 and tumors associated with neurofibromatosis type 1. (AUC=0.57, AUC=0.52 and AUC=0.59 respectively). NSE showed better diagnostic efficiency (AUC=0.68) than the anti-vimentin IgG and anti-vimentin IgM. (AUC=0.63 and AUC=0.56 respectively). Anti-vimentin IgG and IgM antibodies showed higher sensitivity (87.5% and 87.2%) at the cut off value than the NSE (54%). On the contrary, NSE showed higher specificity at the cut off value than both the anti-vimentin IgG and IgM (71% vs. 22.5% and 16% respectively).

CONCLUSIONS: Anti-vimentin IgG and IgM and neuron-specific enolase are relevant markers in investigation of the patients with neurofibromatosis type 1 and associated tumors.

ORIGINAL ARTICLE

Abbreviations

ATP - Adenosine triphosphate
AUC - Area under the curve
ECLIA - Electrochemiluminescence immunoassay
ELISA - Enzyme linked immunosorbent assay
NIH - National Institute of Health
NSE - Neuron-specific enolase
OD - Optical density
ROC - Receiver operator characteristics
SEM - Standard error of mean
VIM - Vimentin

INTRODUCTION

Vimentin is a developmentally regulated intermediate filament protein found in cells of mesenchymal origin. It is involved in the intracellular transport of proteins between the nucleus and plasma membrane. Unlike other intermediate filament proteins, vimentin is expressed, along with desmin, during the early stages of cellular development. Vimentin phosphorylation by a protein kinase causes the breakdown of intermediate filaments and activation of an ATP and myosin light chain dependent contractile event [10,6]. The VIM gene is located on the chromozome 10q13 [1].

Neurofibromatosis von Recklinghausen type 1 (NF-1) is an autosomal dominant disorder with an estimated birth incidence of 1 in 3 000–40 000. The most common clinical picture is characterized by the presence of diagnostic criteria (NIH 1987): café au lait spots, axillary and/or inquinally freckling, neurofibromas and/or plexiform neurofibromas, Lisch nodules, optic glioma, distinct osseous lesions, and first – degree relative with NF-1 [8]. Neoplastic processes associated with NF-1 are usually benign, malignant transformation is described in 4–7% cases [2,11]. The most common tumors associated with NF-1 are neurofibromas and optic gliomas (pilocytic astrocytoma grade I) [9,12,13].

Positive immunohistochemical staining for vimentin antibodies is characteristic for various neoplastic processes, thus, anti-vimentin antibodies are generally used with a panel of other antibodies including those recognising cytokeratins, lymphoid markers, S100 protein, NSE, desmin, and neurofilaments, and they are positive in many tumors. They are also used to their follow-up [3,7].

The aim of the study was to compare the diagnostic ability of serum levels of NSE with anti-vimentin IgG and anti-vimentin IgM antibodies in the patient group with NF-1 and associated tumors (optic glioma and plexiform neurofibroma).

PATIENTS AND METHODS

131 children and adolescents (67 males, mean age 10 years, range 4–19 years; 64 females, mean age 11 years, range 1–20 years) with definite diagnosis of NF-1 according to the NIH criteria were selected for the study. Patients were divided into three groups according to the NF-1 without glioma and plexiform neurofibroma (51 patients), NF-1 with optic glioma (44 patients) and NF-1 with plexiform neurofibroma (36 patients). 40 healthy subjects (20 males, mean age 9 years, range 1–19 years and 20 females, mean age 12 years, range 3–18 years). were taken as control group. All patients gave consent with the investigation. The characteristics of the patients and control group are listed in Table 1. Serum levels of neuron-specific enolase (NSE) were measured using commercially available electrochemiluminescence sandwich immunoassay (ECLIA, Roche) on an Elecsys System 2010. Serum levels of anti-vimentin IgG and anti-vimentin IgM were measured using commercially available indirect heterogenous sandwich enzyme-linked immunoassay with monoclonal antibodies and enzymatic peroxidase detection at 450 nm (ELISA-VIDITEST Anti-Vimentin assay, Vidia; web site: http://www.vidia.cz).

Table 1. The characteristics of the patients and control group.

	Group I Control group	Group II Neurofibromatosis (NF-1)	Group III NF-1 and glioma	Group IV NF-1 and plexiform neurofibroma
N	40	51	44	36
Male	20	25	20	22
Female	20	26	24	14
NSE (mean ± SEM) (µg/l)	13.7±0.5	14.5±0.7	14.0±1.1	12.2±0.8
Anti-vimentin IgG (mean ± SEM) (OD/cut off)	0.57±0.09	0.65±0.08	0.76±0.07	0.62±0.08
Anti-vimentin IgM (mean ± SEM) (OD/cut off)	0.48±0.06	0.59±0.06	0.55±0.06	0.60±0.09

NF-1 – neurofibromatosis type 1; SEM – standard error of mean

Statistical analysis

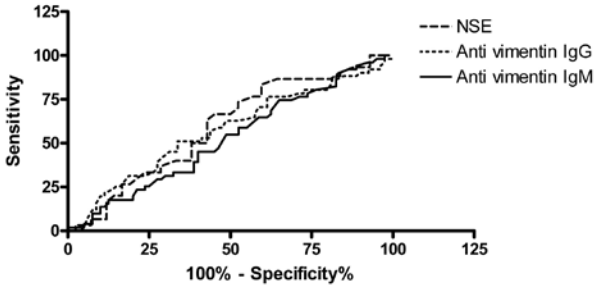
The relationship between patients with NF-1 and associated tumors and diagnostic efficiency were performed by the receiver operating characteristic (ROC) analysis. The differences between control group and patients with all forms of NF-1 were tested either by unpaired t-test, or non-parametric Mann-Whitney U-test, if the data were not normally distributed. A value of $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Anti-vimentin IgG, anti-vimentin IgM antibodies and NSE showed similar ability to distinguish patients with NF-1 and tumors associated with NF-1. (AUC=0.57, AUC=0.52 and AUC=0.59 respectively) (Figure 1). NSE showed better diagnostic efficiency (AUC=0.68) than both the anti-vimentin IgG and IgM antibodies (AUC=0.63 and AUC=0.56 respectively).

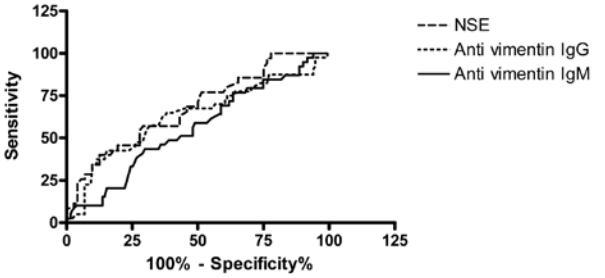
Sensitivity and specificity were calculated based on cut off values as follows: The cut off value for NSE was set at 16 µg/l (upper reference limit). the cut off value for anti-vimentin IgG and IgM was set at the value 1.0. Anti-vimentin IgG and IgM antibodies showed higher sensitivity (87.5% and 87.2%) at the cut off value than the NSE did (54%). NSE showed higher specificity (71%), than both the anti-vimentin IgG and IgM antibodies did (22.5% and 16.5% respectively) (Figure 2).

This paper is one of the first preliminary studies concerning the relevance of new immunochemical markers (anti-vimentin IgG and IgM antibodies) and NSE in patients with neurofibromatosis type 1 and associated tumors. Results show, that both anti-vimentin antibodies and NSE show similar relevance in investigation of tumors associated with neurofibromatosis. These findings correlated with results described in our previous study [4], nevertheless, the findings differed from the expectations that NSE, known as marker of neuronal tissue damage [5] and tumor marker will be more relevant in distinguishing of tumors associated with neurofibromatosis than the anti-vimentin antibodies. However, NSE showed better diagnostic efficiency and thus, ability to distinguish between control group of healthy subjects and patients with neurofibromatosis, than both anti-vimentin antibodies did. These findings were confirmed by the two calculations: First, NSE and anti-vimentin IgG antibodies showed significant differences between patients with all forms of NF-1 and control group (p=0.0006, unpaired t-test and p=0.01, Mann-Whitney U test), although the difference of anti-vimentin IgM antibodies was considered not significant (p=0.27, Mann-Whitney U test). Second, NSE showed high specificity at the cut off value (71%) than anti-vimentin IgG and IgM (22.5% and 16.5% respectively). On the contrary, anti-vimentin IgG and IgM antibodies showed higher sensitivity (87% for both IgG and IgM antibodies) than NSE (54%) and thus, anti-vimentin antibodies are reliable for monitoring the disease. Anti-vimentin IgG and IgM antibodies and NSE are relevant markers in investigation of the patients with neurofibromatosis type 1 and associated tumors. NSE showed better diagnostic efficiency. Anti-vimentin antibodies seems more reliable for monitoring the disease.



	NSE	anti-vimentin IgG	anti-vimentin IgM
AUC	0.59	0.57	0.52

Figure 1. ROC comparison of anti-vimentin antibodies and NSE between patients with neurofibromatosis and patients with tumors associated with NF-1.



	NSE	anti-vimentin IgG	anti-vimentin IgM
Cut off value	16 µg/l	1 (OD/Cut off)	1 (OD/Cut off)
Sensitivity (%)	54	87.5	87.2
Specificity (%)	71	22.5	16
AUC	0.68	0.63	0.56

Figure 2. Assessment of diagnostic efficiency between control group and all patients with NF-1.

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