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Role of neopterin as a biochemical marker for peripheral neuropathy in pediatric patients with type 1 diabetes: Relation to nerve conduction studies

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ABSTRACT

Background: Neopterin, a marker of inflammation and cellular immune response, is elevated in conditions of T-cell or macrophages activation. Diabetic peripheral neuropathy (DPN) is associated with inflammatory/immune processes and therefore, we hypothesized that neopterin could be used as a marker of neuropathy in type 1 diabetes mellitus (T1DM).

Aim: To measure neopterin levels in children and adolescents with T1DM and assess its possible relation to DPN and nerve conduction studies (NCS).

Methods: Sixty patients aged ≤18 years and > 5 years disease duration were subjected to neurological assessment by neuropathy disability score (NDS) and NCS for median, ulnar, posterior tibial and common peroneal nerves. Mean fasting blood glucose, lipid profile, HbA1c, high sensitivity C-reactive protein (hs-CRP) and serum neopterin levels were assessed. Patients were compared with 30 age- and sex-matched healthy controls.

Results: The frequency of DPN according to NDS was 40 (66.7%) patients out of 60 while NCS confirmed that only 30 of those 40 patients had this complication (i.e. 50% out of the total studied patients). Neopterin levels were significantly higher in patients with DPN than those without (median [IQR], 53.5 [35–60] nmol/L versus 17 [13 − 32] nmol/L) and healthy controls (5.0 [3.2–7.0] nmol/L) ($p < 0.001$). Significant positive correlations were found between neopterin levels and HbA1c ($r = 0.560$, $p = 0.005$), serum creatinine ($r = 0.376$, $p = 0.003$), total cholesterol ($r = 0.405$, $p = 0.026$) and hs-CRP ($r = 0.425$, $p = 0.012$) among patients with DPN. Neopterin levels were positively correlated to motor latency of tibial and common peroneal nerves as well as motor and sensory latencies of median and ulnar nerves. Logistic regression analysis revealed that neopterin was a significant independent variable related to DPN (Odds ratio, 2.976). Neopterin cutoff value 32 nmol/L could differentiate patients with and without DPN with 100% sensitivity and 96.7% specificity.

Conclusions: Neopterin could be used as an early reliable serum biomarker for DPN in pediatric patients with T1DM.

1. Introduction

The increasing incidence of type 1 diabetes mellitus (T1DM) in many countries challenges health systems because the disease is presently incurable with no known method of prevention [1]. Diabetic peripheral neuropathy (DPN) is the most common chronic complication of diabetes mellitus, with an incidence rate of about 50% [2–4]. There is a progressive increase in the DPN prevalence over time, in particular its subclinical stages [5]. The high rates of DPN among youth with diabetes are a cause of concern and suggest a need for early screening and better risk factor management [6]. However, there are insufficient data on the prevalence and predictors of DPN among the pediatric population [7–9].

DPN commonly develops insidiously, with various clinical manifestations. In the early stages, diagnosis is difficult as there are no symptoms [10,11]. Once symptoms appear, there are few effective therapeutic strategies [12]. Therefore, mere clinical evaluation is not sensitive enough to diagnose DPN [9]. Early discovery and diagnosis are extremely important [13]. Fortunately, increasing use of electrophysiological techniques that allow the identification of sub-clinical pathological changes has made early diagnosis of DPN possible [14].

DPN is associated with inflammatory/immune processes [15].

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Neopterin, a pyrazino-pyrimidine compound, is synthesized by monocytes and macrophages in response to Interferon (IFN-γ) produced by activated T cells. Neopterin levels are elevated in conditions of T-cell or macrophages activation [16]. It enhances macrophage cytotoxicity through its interactions with reactive oxygen, nitrogen, and chloride species [17].

Neopterin levels are elevated in several conditions including autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis [18–20]. Neopterin has emerged as a novel independent predictor of fatal ischemic heart disease in type 2 diabetes mellitus [21] and was also elevated in gestational diabetes mellitus [22]. High levels of neopterin were found in cerebrospinal fluid of patients with neurological diseases [23,24]. Although neopterin levels were significantly elevated in patients with peripheral neuropathies such as Guillain-Barré syndrome [25] and has been reported as a marker of disease progression and complications in diabetes [26]; yet, its clinical relevance and relation to DPN remains largely unknown.

To the best of our knowledge, up till now, there are no published data on serum levels of neopterin in pediatric patients with T1DM. Therefore, to test the hypothesis that neopterin could be a potential biomarker for DPN in an early stage, we measured neopterin levels in children and adolescents with T1DM and assessed its relation to glycemic control, clinically assessed DPN and nerve conduction studies (NCS).

2. Materials and methods

This cross sectional study included 60 children and adolescents with type 1 diabetes (aged ≤ 18 years with at least 5 years disease duration) attending the Pediatric Diabetes Clinic, Pediatric Hospital, Ain Shams University. Patients were defined according to the criteria of American Diabetes Association [27]. All patients did not have symptoms of neuropathy and were on insulin therapy using human insulin with a mean dose of 1.8 ± 0.42 IU/kg/day. Exclusion criteria were the presence of any clinical or laboratory evidence of chronic infection, any neurological disease, history of drugs that may affect neural function, nutrition deficiency, liver or renal dysfunction, connective tissue disease, or other autoimmune disorders, and any other conditions that could influence high-sensitivity C-reactive protein (hs-CRP). Patients with hs-CRP > 10 mg/L were excluded. Moreover, other microvascular complications than neuropathy (nephropathy and retinopathy) and macrovascular diabetic complications were also excluded. The 60 studied patients were recruited from of a total of 96 consecutive patients attended our clinic during the study period after fulfilling of inclusion criteria while 36 patients were excluded due to the above-mentioned exclusion criteria. Another group of 30 age-, sex- and pubertal stage-matched healthy volunteers were enrolled as controls. An informed consent was obtained from each patient or control subject or their legal guardians before enrollment into the study. This study was approved by the local ethical committee of Ain Shams University.

2.1. Detailed medical history and examination

The studied patients were subjected to detailed medical history and thorough clinical examination with special emphasis on age of onset of diabetes, disease duration, insulin therapy and chronic diabetic complications (nephropathy, neuropathy, retinopathy or cardiovascular ischemic events). Anthropometric measurements were recorded and body mass index (BMI) was calculated. Pubertal stage was determined according to Tanner's classification [28]. Blood pressure was measured after a 5-minute rest in the seated position using mercury sphygmomanometer. If it was greater than 90th percentile for age and sex, the blood pressure was repeated twice for the validity of the reading. Standard deviation scores (SDS) for mean blood pressure were calculated according to the report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents [29]. Fundus examination was performed through dilated pupils using 90-diopter Volk lens and biomicroscope to exclude retinopathy.

2.2. Neurological assessment

The simple rapid bedside neuropathy disability score (NDS) was adopted as a screening tool for DPN. The NDS was derived from examination of vibration perception (by means of a 128-Hz tuning fork), pinprick and temperature perceptions in the great toe, and the presence or absence of ankle reflexes. The sensory modalities were scored and a score above two was defined as clinical peripheral neuropathy [30].

All patients underwent NCS using (EMG/NLG/EP-system type Topas; Schwarzer, Munich, Germany). Motor nerve conduction of median nerve, ulnar nerve, posterior tibial nerve and common peroneal nerve were measured separately as previously described [31]. Considering the fact that sensory affection of lower limbs usually occurs early in diabetic patients even without symptoms [13,32] and disease duration in our studied patients was at least 5 years; therefore, we first performed motor nerve conduction analysis for lower limbs and if it was normal, we proceeded to examine sensory nerve conduction of lower limbs.

Each patient lay on a bed in a quiet room (22–25 °C) with limbs relaxed. For motor conduction of ulnar nerve, the active electrode was placed on abducto digiti minimi on a point midway between the distal wrist crease and the crease at the base of the fifth digit at the junction of dorsal palmar skin. The reference electrode was on the fifth digit. For median motor conduction, the active electrode was placed one and half the distance (prominence of abductor polices brevis) between the metacarpophalangeal joint of thumb and the midpoint of distal wrist crease. The reference was on the distal phalanx of thumb. For motor conduction of common peroneal nerve, the active electrode was placed over the extensor digitorum brevis and the reference was just below the 5th toe while for the posterior tibial nerve, the active electrode was placed 1 cm behind and 1 cm below the navicular tuberacle at medial side of foot on abductor hallucis muscle and the reference was placed on the large toe. In all motor conduction tests, the ground wire was placed between the stimulus and recording electrodes. For sensory nerve conduction tests, a ring electrode was placed around the end of the fingers, which is the distal end of the sensory nerve, and used to stimulate the median and ulnar nerves. Using standard nerve conduction techniques, amplitudes, velocities, and latencies of both sensory and motor nerves were recorded. Control values corresponded to the standards of electromyography for age and gender [33–36].

2.3. Laboratory investigations

Fasting blood glucose (FBG) levels, fasting lipid profile, routine liver and renal functions as well as hs-CRP were measured using Cobas Integra 800 (Roche Diagnostics, Mannheim, Germany). FBG was assessed during regular clinic visits with calculations of the mean values during the last 6 months prior to the study including the level of a blood sample at the start of the study. Dyslipidemia was defined if at least one of the following was present; serum total cholesterol ≥ 200 mg/dL, low-density lipoprotein (LDL) cholesterol ≥ 100 mg/dL, high-density lipoprotein cholesterol (HDL) < 40 mg/dL, or serum triglyceride ≥ 150 mg/dL [37]. Further analysis was done after controlling for age and pubertal stage to avoid differences in lipid values [38]. Assessment of mean HbA1c% in the year preceding the study was performed using D-10 (BioRad, France). To exclude nephropathy, urinary albumin excretion was measured in an early morning urine sample as albumin-to-creatinine ratio by an immuno-nephelometric method [39,40]. Determination of serum levels of neopterin was done by enzyme linked immunosorbent assay (ELISA) using kit supplied by IBL International GmbH, Hamburg, Germany according to the manufacturer's instructions.