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Plasma Neopterin Level as a Marker of Peripheral Immune Activation in Amnestic Mild Cognitive Impairment and Alzheimer's disease

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Abstract

Objective—Alterations of the immune system play important roles in Alzheimer's disease (AD). The primary purpose of this study was to compare plasma levels of neopterin, a marker of cellular immune activity, in amnestic Mild Cognitive Impairment (aMCI), early (mild to moderate) AD, and cognitively normal controls (NC). In addition, correlation of plasma neopterin with interferon-gamma (INF- γ) and Interleukin-6 (IL-6) were also examined.

Methods—Plasma samples from patients with mild to moderate AD (N=35), aMCI (N= 24), and cognitively normal controls (N = 29) were obtained from the Johns Hopkins Alzheimer's Disease Research Center. Plasma neopterin, INF- γ , and IL-6 levels were measured using commercially available ELISAs. Multiple linear regression was performed to study differences in the baseline neopterin levels between normal, aMCI and AD patients. Pearson correlation coefficients were estimated for neopterin and INF- γ and IL-6 levels. All analyses were conducted using SAS and GraphPad softwares.

Results—AD subjects had significantly higher neopterin values compared to aMCI ($\beta = 0.202$, $p = 0.004$) and normal ($\beta = 0.263$, $p = 0.0004$) subjects. There was no statistically significant difference between normal and aMCI. A significant association between INF- γ ($r = 0.41$, $p < 0.0001$) and IL-6 ($r = 0.35$, $p = 0.0006$) levels were found.

Conclusions—Our study demonstrates that peripheral immune response may be stronger in later stages of AD pathophysiology, when dementia has developed.

Keywords

Alzheimer's disease; Amnestic Mild Cognitive Impairment; Inflammation; Neopterin

Introduction

Inflammation is closely associated with the progression of neurodegenerative conditions such as Alzheimer's disease (AD) (Frank-Cannon et al. 2009). There is growing evidence in AD that neurotoxicity is mediated by inflammation (McGeer, McGeer 2001) including alterations in the cellular immune response (Britschgi, Wyss-Coray 2007). The activation of macrophages which produce cytokines and other inflammatory mediators including neopterin is one of the major cellular immune responses (Hoffmann, Wirleitner & Fuchs 2003).

Neopterin is formed in human monocytes/macrophages as a cleavage byproduct of guanosine triphosphate (GTP) by GTP-cyclohydrolase I. GTP-cyclohydrolase I activity is enhanced upon stimulation by interferon-gamma (INF- γ) (Schoedon et al. 1987), which is mainly produced by activated T-cells (Hamerlinck 1999a). Cleavage of GTP results in 7,8 dihydroneopterin triphosphate (NH₂TP), which is further processed by 6-pyrovoyl tetrahydropterin synthase (PTPS) into 6-pyrovoyl tetrahydropterin (Hoffmann, Wirleitner & Fuchs 2003). However, due to lower levels of PTPS in human monocytes and macrophages, more NH₂TP are processed to yield neopterin than 6-pyrovoyl tetrahydropterin in humans compared to lower mammals (Schoedon et al. 1987, Werner et al. 1990). Clinically, increased levels of neopterin can be detected in blood, cerebrospinal fluid (CSF) and urine taken from patients with many conditions in which the cellular immune system is activated (Hamerlinck 1999b).

Previous studies have reported higher levels of peripheral neopterin in persons with AD compared to cognitively normal controls (Leblhuber et al. 1999, Hull, Pasinetti & Aisen 2000, Casal, Robles & Tutor 2003, Blasko et al. 2007) or to individuals with other brain diseases (Casal, Robles & Tutor 2003, Mehta et al. 2005). Increase in neopterin level has also been shown to correlate with greater cognitive decline in advanced AD patients (Blasko et al. 2007). However, there is limited information on the association between neopterin and earlier stages of AD. Two of the studies cited above included subjects who were in advanced stages of AD, as determined by the Mini-Mental State Examination (MMSE) (Leblhuber et al. 1999, Hull, Pasinetti & Aisen 2000). In addition, previous studies of MCI participants either grouped them with AD subjects (Frick et al. 2006, Greilberger et al. 2008), or involved a small number of subjects (Frick et al. 2006).

Given the evidence for the role of inflammation in the neurotoxicity of AD (McGeer, McGeer 2001), peripheral inflammatory markers have been extensively studied as possible biomarkers of inflammation and predictors of cognitive decline. They may be markers of inflammatory processes that accelerate the AD pathology or be the result of the disease process (Wyss-Coray 2006). Although there are many studies examining the levels of different cytokines and other inflammatory markers (Swardfager et al. 2010), their limitation is the biological instability and short half-life (Hagberg et al. 2010). Thus, the advantages of measuring neopterin include its stability in biological fluids, diffusibility and long half-life (Hagberg et al. 2010).

The primary aim of our study was to compare plasma neopterin levels in aMCI and early AD to that in cognitively normal controls. Since persons with aMCI are at high risk of progressing to AD (Petersen 2010), it is important to determine if there is an inflammatory phenotype that is specific to a certain stage of AD pathology (Magaki et al. 2007). Secondly, we also examined the association of plasma neopterin levels with two other inflammatory markers: (1) INF- γ which stimulates neopterin production and (2) IL-6, a marker of systemic inflammation.

Methods

Our study comprised 91 participants evaluated in the Clinical Core of the Johns Hopkins Alzheimer's Disease Research Center (JHADRC) study between 2006 and 2009. There were 27 individuals with aMCI, 24 with early (mild to moderate) AD, and 30 with normal cognition (Table 1). Participants with aMCI met Petersen criteria (Petersen 2004), had a Clinical Dementia Rating (CDR) = 0.5, and Mini-Mental Status Exam (MMSE) score ≥ 24 . A diagnosis of probable AD was based on NINCDS-ADRDA criteria (McKhann et al. 1984). Cognitively normal controls (NC) had CDR of 0.0, MMSE > 28 , and no reported memory impairments. Annual cognitive diagnoses for JHADRC participants were derived from consensus conferences based on structured histories for participants and informants who knew them well, followed by medical, psychiatric, neurological, and neuropsychological examinations. The study was approved by a Johns Hopkins Institutional Review Board, and all participants, or a surrogate, gave informed consent.

Non-fasting blood was collected from the JHADRC participants at each visit. All blood draws and processing followed established JHADRC protocols. Blood samples used in these analyses were collected in EDTA polypropylene tubes for plasma, centrifuged at 3000 rpm for 15 minutes at 4°C, divided into 0.5 ml aliquots, and stored at -80°C until analysis.

Plasma neopterin was measured using a commercially available competitive ELISA (ALPCO Diagnostics, Inc., Salem, NH). Briefly, microtiter plates coated with anti-neopterin antibodies were incubated with a 150 μl mixture of plasma and neopterin/alkaline phosphatase conjugate (1:3 ratio) at room temperature for 2 hours in the dark. After washing with BRAHMS wash solution 4 times, the plates were incubated with 100 μl of dissolved substrate (4-nitrophenyl phosphate) at room temperature for 30 minutes. After this incubation, 100 μl of stop solution was added to each well, and the plate was read on spectrophotometer at 405nm. This immunoassay has an interassay coefficient of variance of 5.50% (Spencer et al. 2010). Plasma INF- γ and IL-6 levels were measured using a sandwich-based ELISA human cytokine kit (Meso Scale Discovery, Gaithersburg, MD).

The neopterin, IL-6 and INF- γ levels were log transformed prior to analyses due to the skewness of the data. Multiple linear regression models were estimated to compare neopterin levels between aMCI, AD, and normal participants. The final multiple linear regression model was adjusted for age, gender, race, body mass index (BMI), and ApoE4 status based on our own analysis and previous literature (Spencer et al. 2010). Pearson correlation coefficients were estimated between plasma levels of neopterin with IL-6 and INF- γ levels. Analyses were conducted using SAS (SAS Institute, Inc., Cary, NC) and GraphPad Prism version 5.00 for Window (GraphPad Software, San Diego, CA, USA).

Results

The baseline characteristics of these subjects are described in Table 1. Mean log plasma neopterin levels varied by diagnosis: NC 2.06 ± 0.22 nM, aMCI 2.19 ± 0.23 nM, and AD 2.27 ± 0.36 nM (Figure 1). In multiple linear regression model, AD participants had significantly higher plasma neopterin levels than did those with aMCI ($\beta = 0.202$, $p=0.004$) and NC ($\beta = 0.263$, $p<0.0004$); there were no significant differences in neopterin levels between aMCI and NC subjects. Plasma neopterin levels were significantly correlated with INF- γ ($r= 0.41$, $p<0.0001$) and IL-6 ($r= 0.35$, $p=0.0006$) levels (Figure 2).

There was an association between age and plasma neopterin levels ($\beta = 0.017$, $p<0.0001$), consistent with previous studies (Hull, Pasinetti & Aisen 2000, Spencer et al. 2010). However, there were no significant associations between plasma neopterin levels and years of education, BMI or APOE 4 genotype (data not shown).

Discussion

In the present study, plasma neopterin levels in early AD were significantly higher than in aMCI and NC subjects. This expands previous studies demonstrating higher neopterin levels in advanced AD compared to early AD (Leblhuber et al. 1999, Casal, Robles & Tutor 2003) or NCs (Leblhuber et al. 1999, Hull, Pasinetti & Aisen 2000, Casal, Robles & Tutor 2003). Notably, mean log neopterin levels in aMCI differed compared to NCs, but not significantly, which suggests that cellular immune response may be stronger in later stages of AD when dementia has developed. However, the lack of significance may also be due to lack of power from small sample size.

Our results are consistent with Frick et al. who reported significantly higher serum neopterin levels in AD compared to MCI (Frick et al. 2006). However, the study participants had more advanced AD, and there was no comparison with NC subjects. A different study by Greilberger et al did not show differences in serum neopterin between AD and NC; however, AD participants were grouped together with MCI cases (Greilberger et al. 2008). As our study did not show significant differences in the neopterin levels between aMCI and NCs, combining AD and MCI subjects may have influenced their finding.

One of the hypothesized ways in which AD neuropathology can be reflected in the systemic immune system is the efflux of peptides such as amyloid-beta ($A\beta$) and inflammatory mediators from the brain into the periphery (Britschgi, Wyss-Coray 2007). $A\beta$ peptide (Craft, Watterson & Van Eldik 2006), a major structural component of amyloid plaques in AD (Hardy, Selkoe 2002), activates microglia that produce cytokines and other toxic inflammatory mediators (McGeer, McGeer 2010). These CNS peptides and inflammatory mediators may in turn activate the systemic cellular immune system including peripheral macrophages that produce neopterin. Therefore, higher neopterin levels observed in AD group may be partly due to the progression of AD pathology and neuroinflammation. One of the proposed mediators of systemic inflammation is $A\beta$ in the brain that may have effluxed from CNS (Britschgi, Wyss-Coray 2007, Oh, Troncoso & Fangmark Tucker 2008) to stimulate increased production of neopterin outside the CNS. We previously demonstrated in the same sample that plasma $A\beta$ levels did not differ in these diagnostic groups (aMCI, AD and NC) (Oh et al. 2010). Therefore, plasma $A\beta$ is less likely to be the cause for the increase in neopterin level in this study. This is also consistent with another study that showed no correlation between serum neopterin and $A\beta$ levels in patients with Down syndrome (DS) (Mehta et al. 2005). However, we cannot rule out the possibility that other inflammatory mediators exiting from the brain (Banks, Kastin & Broadwell 1995) may be contributing to the peripheral cellular immune activation and hence higher neopterin levels.

Although CSF markers of neuroinflammation including neopterin were not measured in this study, elevated levels of CSF neopterin have been detected in different CNS disease processes (Hagberg et al. 2010, Millner et al. 1998). Correlations of CSF and peripheral (blood) neopterin levels appear to vary depending on the disease processes, from those that correlate strongly such as in patients with HIV infection (Hagberg et al. 2010) to those that correlate poorly such as in pediatric patients with CNS infection (Millner et al. 1998). One study which examined CSF neopterin levels in AD compared to normal controls did not find differences between the two groups (Engelborghs et al. 1999). However, peripheral neopterin levels were not examined in this study, and additional studies specifically examining the association of CSF and peripheral neopterin levels in AD would be needed in the future.

It is also possible that neopterin is a marker of peripheral systemic inflammation that does not directly depend on the efflux of inflammatory mediators from the CNS (Swardfager et

al. 2010). Studies have shown that systemic inflammation is associated with cognitive decline in older adults (Yaffe et al. 2003), and increased risk of the development (Tan et al. 2007) or progression of AD (Blasko et al. 2007, Holmes et al. 2009). In our study, we demonstrate a significant correlation of neopterin with INF- γ , which is mostly produced by T-cells, and with another marker of systemic inflammation, IL-6, further supporting this concept.

Neopterin itself may also have a direct neurotoxic effect as its derivatives may act as key regulators of macrophage cytotoxic effector functions by enhancing the activity of reactive oxygen, nitrogen and chlorine species (Weiss et al. 1993). In addition, neopterin synthesis by macrophages is associated with the production of reactive oxygen species (ROS) (Weiss et al. 1993), expression of NF- κ B (Wirleitner et al. 1997), and the release of cytokine and inflammatory mediators.

One of the systemic infections that may increase neopterin levels is cytomegalovirus (CMV) infection. CMV-antibody concentrations have previously been shown to significantly correlate with neopterin levels in AD, as the production of INF- γ from senescent CMV-specific T cell clones may be increasing neopterin levels (Blasko et al. 2007). However, there was no significant correlation between baseline CMV-antibody concentration and CERAD z-scores, whereas there was a significant correlation between baseline neopterin levels and changes in verbal fluency scores, and between changes in neopterin levels and changes in MMSE and combined CERAD z-scores. CMV infection is highly prevalent world-wide, affecting on the order of 86% of individuals in the 70–79 age group (Wang et al. 2010). In addition, at least one study has shown no difference in CMV antibody titers between AD patients and NCs (Renvoize, Hambling 1984). Nevertheless, the association between neopterin and CMV remains an interesting matter as studies have shown relationship between CMV infection and frailty (Wang et al. 2010, Schmaltz et al. 2005). As frailty is associated with functional decline, institutionalization and death (Bandeem-Roche et al. 2006), it is possible that neopterin may be a marker of a subset of AD subjects who have poor physiological reserve and prognosis.

In this study, we report that plasma neopterin levels are elevated in AD compared to aMCI and NCs. A limitation of the study is its cross-sectional nature which does not allow determination of temporal relationships or of change in plasma neopterin levels with disease progression. In addition, as the study analyzed stored samples, we could not confirm that participants were not actively infected in ways that would account for variations in neopterin levels. The strengths of the study include examining the plasma neopterin levels in early AD and aMCI who are at high risk of progressing to AD. In addition, longitudinal follow-up of this cohort will enable examination of neopterin levels and other inflammatory markers in relationship to trajectory of cognitive changes. We are currently investigating whether neopterin is a useful marker in identifying those among aMCI who may have a faster decline or earlier conversion to AD.

Acknowledgments

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Figure 1. Box and whisker plot of neopterin values in amnesic Mild Cognitive Impairment (aMCI), early AD and NC subjects

Multiple linear regression of log plasma neopterin values showed significantly higher neopterin values in AD compared to NC ($p = 0.004$) and aMCI ($p = 0.0004$) subjects. There were no statistically significant differences between normal and MCI. Adjusted for age, gender, race, BMI and ApoE4.

Figure 2. Plasma neopterin levels are associated with IFN- γ and IL-6

Plasma INF- γ , which enhances neopterin production, correlates significantly with plasma levels of neopterin ($r = 0.41$, $p < 0.0001$). Plasma neopterin also has a significant correlation with IL-6 ($r = 0.35$, $p = 0.0006$), which is associated with Alzheimer's disease (Swardfager et al. 2010).

Table 1

Demographic/cognitive characteristics and neopterin levels of amnestic mild cognitive impairment (aMCI), normal control (NC), and early Alzheimer's disease (AD) groups

	NC n = 30	aMCI n = 27	AD n = 34
Demographics (mean ± SD) ^Δ			
Age in years [*]	72.62 (7.71)	78.22 (8.08)	73.18 (8.86)
Gender (male) (no, %) [*]	6 (20.69)	13 (48.15)	16 (47.06)
Race (no, %) [*]	9 (30)	9 (33.33)	6 (17.65)
Education [†]	15.27 (2.56)	15.44 (2.83)	16.41 (2.61)
BMI [‡]	29.07 (8.10)	27.82 (5.86)	25.30 (4.09)
ApoE4 ⁺ (no, %) ^{***§}	8 (28.57)	11 (40.74)	23 (67.65)
Cognitive Measurement/Disease Stage (mean ± SD)			
MMSE ^{****§}	29.77 (0.63)	28.44 (1.45)	24.06 (3.14)
Total CDR ^{****}	0.02 (0.09)	0.52 (0.10)	1.10 (0.46)
Sum of Boxes CDR ^{****}	0.03 (0.13)	1.69 (1.00)	6.59 (2.57)
Neopterin levels (mean ± SD)			
Neopterin (nM) ^{***}	8.05 (1.66)	9.15 (2.35)	10.35 (4.33)
Log-neopterin ^{**}	2.06 (0.22)	2.19 (0.23)	2.27 (0.36)

^{*} P 0.05,

^{**} P 0.01,

^{***} P 0.001,

^{****} P 0.0001;

^Δ (mean ± SD) unless noted as (no, %);

Race – number of African Americans;

[†] Education – number of years;

[‡] BMI-Body Mass Index [(weight lb) / (height inches)²] × 703;

[§] ApoE4⁺ – one or two copies of ApoE4.