Title

Cognitive ability in Down Syndrome and its relationship to urinary neopterin, a marker of activated cellular immunity

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Abstract

Background: Neopterin is an unconjugated pteridine that is secreted in large quantities by activated macrophages and can be used as a clinical marker of activated cellular immunity and oxidative stress. We aimed to investigate whether urinary neopterin levels are associated with cognitive function in people with Down syndrome (DS).

Methods: Out of 32 adults with DS who originally participated in a longitudinal study, 25 were followed up at 4 years. Informants rated their adaptive behavior (ABAS) and the adults with DS attempted assessments of language skills and memory at both baseline and follow-up time points (Modified Memory Object Task, MOMT), and receptive vocabulary (British Picture Vocabulary Scale, BPVS).

Results: Neopterin/creatinine levels were negatively correlated with change in the MOMT total score (Spearman's Rho = -0.517, p =0.020) and change in the MOMT delayed recall score (Spearman's Rho = -0.577, p =0.008) over time, i.e. higher neopterin/creatinine level was associated with worse performance on a test of cognitive ability over time.

Conclusion: Urine neopterin may have potential as a biomarker for memory decline in Down syndrome, and could potentially also help to track progression of mild cognitive impairment (MCI) to Alzheimer's disease in other high risk populations.

Key words

Words 1,915

Tables 1

1. Introduction

Oxidative stress is related to the imbalance between oxidant production and the organism's defenses against its potential damage. In oxidative stress, the organism lacks the ability to detoxify the system of reactive oxygen species (ROS) and repair the damage they cause. Levels of ROS above the clearance capacity of the body can cause mitochondrial dysfunction, cellular damage, and cell death [1]. Oxidative stress has been implicated in the pathogenesis of many diseases, including Alzheimer's disease (AD) [2,3].

In people with Down syndrome (DS), the most common genetic cause of intellectual disability (ID), the prevalence of AD increases significantly with age [4]. By the age of 50, almost all people with DS have neuropathological [5-7] and neuroimaging changes characteristic of AD [5, 6]. Although this may be in large part due to the triplication of the APP gene in DS, increasing evidence suggests that oxidative stress and inflammation may also play a role in AD pathogenesis associated with the syndrome [8].

Neopterin is an unconjugated pteridine that is secreted in large quantities by activated macrophages and can be used as a clinical marker of activated cellular immunity [9]. Plasma neopterin levels have been found to be higher in patients with AD [10, 11] and people with DS [12], with [13] or without [14, 15] dementia. Moreover, urinary neopterin levels correlate with cerebrospinal fluid neopterin levels in specific patient populations, such as multiple sclerosis patients with positive oligoclonal bands [16].

We aimed to investigate whether urinary neopterin levels are associated with cognitive function in people with DS and whether these levels correlate with isoprostane 8,12-iso-iPF2alpha (iPF2alpha), a urinary marker of oxidative stress.

2. Methods and Materials

2.1. Study population, iPF2alpha levels, neopterin levels and psychometric measurements.

We used a within-syndrome design to examine the association between urinary markers of oxidative stress and changes in cognitive functioning over time in adults with DS. We have previously established a cohort of 32 individuals with DS who underwent cognitive testing and donated urine samples for iPF2alpha [17]. The patients were followed-up 4 years later [18] when they also donated a urine sample for iPF2alpha [19] and neopterin analysis. Urine spot samples were collected in the morning and were stored within 6 hours of collection at -80˚C.

Methods for measuring iPF2alpha (corrected for creatinine concentrations) have been described elsewhere for both the baseline [17] and the follow-up time points [19].

Urine neopterin levels were measured at follow-up. Urine stored at -80ºC was initially allowed to thaw at room temperature in the dark, mixed thoroughly by repeated inversion, and diluted 1/100 with mobile phase (15mM Potassium Phosphate; pH 6.4). Five hundred microlitres of diluted urine was then processed through a 0.45μM RC membrane (4mm) syringe filter. Each sample was analysed in triplicate with an injection volume of 20μl and the coefficients of variation for

neopterin were 4.4% (intra-assay) and 8.6% (inter-assay). Each sample was analysed on an Agilent Technologies 1100 system at a flow rate of 1 mL/min using a reversed-phase SphereClone ODS(2) (4.6 x 250 mm, 5 μm) column coupled to a SecurityGuard guard cartridge (Phenomenex). Fluorescence detection of neopterin was performed using a 1260 Infinity Fluorescence Detector with an excitation and emission wavelength of 353 nm and 438 nm, respectively. Creatinine was detected using an Agilent 1100 series UV detector with a wavelength of 235 nm. The concentration of neopterin in the urine was then normalised per mole of creatinine.

Details of the psychometric assessments and population characteristics have also been described elsewhere [17 – 19]. In brief, informants (who were in most cases family members) completed a measure of adaptive behavior (Adaptive Behaviour Assessment Scale; ABAS [20]) and a dementia screen (the cognitive scale of the Dementia Scale for people with Learning Disabilities; DLD [21]). The DLD identifies individuals who may benefit from a clinical assessment for dementia, but does not provide a formal diagnosis. If a difference of 7 points or more on the sum of cognitive scores (i.e. short- and long-term memory and orientation) and/or 5 points or more on the sum of social scores (i.e. speech, activity, mood, behaviour and practical skills) is found between the baseline assessment and subsequent assessments, assessment for dementia should be considered [22].

Participants who were able to, completed a measure of receptive vocabulary (British picture vocabulary scale; BPVS II [23]), and a modification of an object memory task (MOMT) based on the Fuld object memory test [24, 25]. We modified the task by reducing the number of objects to 6, and the number of trials to two.

2.2. Ethics statement and consent procedures

The study was approved by the National NHS research ethics committee and we followed the Mental Capacity Act, UK (2005) if participants did not have the capacity to consent for themselves, by gaining agreement from carers. Written informed consent or carer's agreement for participation was obtained for all participants.

2.3. Data analyses

Data was analysed with Statistical Package for the Social Sciences (SPSS) [26]. Mann Whitney U tests were used to compare scores between groups. Correlations between neopterin levels and baseline factors were examined using Spearman's correlations. Correlations between the changes in psychometric and functional assessments scores (BPVS, ABAS, MOMT) were examined using Spearman's correlations. The significance level was set at 0.05.

3. Results

3.1. Study population

The original cohort consisted of 32 individuals with DS. Two of the original participants refused to participate again, and four were lost to follow-up. All of the remaining 26 individuals were followed up. Twenty-five urine samples were available for neopterin analysis. The 25 participants (52% females) had a mean age of 36.6 $\pm$ 7.2 years. None was diagnosed with Alzheimer's disease at baseline. At follow up, 4 of the 26 individuals screened above threshold on DLD, but did not have a clinical diagnosis of AD and upon assessment did not present with sufficient features for a DSM-IV or ICD10 diagnosis.

3.2. Neopterin, creatinine and iPF2alpha measurements

At follow-up, urinary neopterin levels ranged from 0.13 to 6.49 µmol/ml (mean 1.79 ± 1.28), urinary creatinine levels ranged from 1.23 to 37.50 mmol/ml (mean 12.75 ± 9.38) and neopterin levels adjusted for creatinine ranged from 76.82 to 434.89 µmol/mol creatinine (mean 162.91 ± 84.32). No significant correlation was observed between age and urinary neopterin (Spearman's rho 0.107, p=0.610) or urinary creatinine (Spearman's rho 0.083, p=0.694).

Data on urinary iPF2alpha were available for 24/26 patients at baseline and for 25/26 patients at follow-up. At baseline, the range of iPF2alpha was 0.16 to 3.18 ng/mg creatinine (mean 1.40 ± 0.81) and at follow-up 0.28 to 5.27 ng/mg creatinine (mean 1.35 ± 1.22). For the 23/26 patients that had measurement on both time points the change between iPF2alpha levels at follow up and at baseline ranged from -2.60 to +4.30 ng/mg creatinine (mean 0.007 ± 1.60).

3.3. Relationship between neopterin and urinary iPF2alpha

Neopterin/creatinine levels were not correlated significantly with the Ipf2alpha/creatinine levels (Spearman's 0.312, p=0.129). However, neopterin/creatinine levels were positively correlated with the change in urinary iPF2alpha/creatinine levels over the 4-year period (Spearman's rho 0.445, p=0.034).

3.4. Relationship between urinary neopterin/creatinine and cognitive ability changes

Table 1 lists the correlations between levels of neopterin/creatinine and performance over 4 years on tests of functional ability, memory or language ability. There were no statistically significant correlations between levels of

neopterin/creatinine and change in ABAS total score, change in ABAS functional academic score, or change in BPVS score. However, neopterin/creatinine levels were negatively correlated with change in the MOMT total score (Spearman's Rho = -0.517, p =0.020) and change in the MOMT delayed recall score (Spearman's Rho = -0.577, p =0.008).

4. Discussion

We have previously shown that change in urinary iPF2alpha/creatinine over time is associated with memory decline in people with DS, suggesting that sequential measurements of urinary iPF2alpha may have potential as biomarker for cognitive decline and progression to AD in this population [19]. In the present study, we have explored the relationship between cognitive change over time in adults with DS and urinary neopterin, a marker of immune activation, and its relationship to oxidative stress. Compared to other markers of activated cellular immunity and oxidative stress, such as isoprostanes, neopterin analysis is relatively low cost which is an advantage in both the clinical and the research setting.

Our results suggest that neopterin/creatinine levels correlate with cognitive ability over time suggesting that urinary neopterin may be an early marker of cognitive decline in DS. In our study, higher neopterin/creatinine level was associated with worse performance on a test of cognitive ability over time (positive difference in the MOMT score means improvement and negative difference in the MOMT score means deterioration). This finding is consistent with other studies where it has been shown that plasma neopterin levels are higher in demented individuals with DS [13] and that a high plasma neopterin level is an independent determinant of the risk of

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dementia in persons with Down syndrome [27]. Plasma neopterin has been shown to be increased in healthy adults with DS compared to age and sex matched healthy controls [15]. Our results extend these findings by demonstrating that neopterin levels may be associated with memory decline before the onset of dementia in this population.

It has been suggested that oxidative stress is an important consequence of the specific biology of DS, and is likely to play a role in some of the co-morbidities associated with DS, including the tendency to develop AD [28]. Oxidative damage may lead to enhanced amyloid beta peptide (Aβ) production [29-33]. Although the precise mechanism is still unknown it has been shown that oxidation of proteins is an early event in DS and might contribute to neurodegenerative phenomena [34].

Although neopterin may play a role as a measure of oxidative stress, since the amount of neopterin produced by activated monocytes/macrophages correlates with their capacity to release ROS [35], neopterin is primarily a measure of immune response [9, 35-37]. Although oxidative damage may be one of the causes of inflammation or immune response, it is not necessarily the only cause and, therefore, the relationship between neopterin and markers of oxidative stress may be partially related to other, unmeasured factors. This may account for our observation that there was not direct relationship between neopterin and iPF2alpha levels; however, there was a relationship between neopterin levels at follow-up and a change in iPF2alpha levels between baseline and follow-up .

 The strength of our study is that it is – to our knowledge – the first to show that urinary neopterin may be used as a biomarker of immune activation due to the

pathogenic processes involved in AD in DS, including the effects of oxidative stress, and which correlates with cognitive decline over time in this population.

Our results should be interpreted with some caution given the limitations of our design. Firstly, our sample size was relatively small, and our results need to be confirmed with a replication larger study. As we have not measured the neopterin level at baseline we can only indirectly correlate the neopterin level with cognitive decline over time. A prospective cohort study is needed to confirm our findings.

In conclusion, for the first time, it is suggested that urinary neopterin is correlated with cognitive decline DS individuals before the diagnosis of AD. This finding strengthens the evidence that peripheral neopterin levels may have potential as a biomarker in DS, and in AD. The evidence for the role of immune response and oxidative stress in DS is growing and also suggests potential treatment strategies to reduce the high risk for AD in this population.

Acknowledgments

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