The Egyptian Journal of Medical Human Genetics (2013) **14**, 49–53

Ain Shams University

The Egyptian Journal of Medical Human Genetics

www.ejmhg.eg.net
www.sciencedirect.com

ORIGINAL ARTICLE

Markers of neural degeneration and regeneration in Down syndrome patients

Iman Ehsan Abdel-Meguid [a], Ekram Abdel-Salam [a,*], Doaa M Abdel Latif [b], Soheir Korraa [c], Amal Ismaiel [b]

[a] *Department of Pediatrics, Genetic Unit, Faculty of Medicine, Cairo University, Egypt*
[b] *Department of Biochemistry, Faculty of Pharmacy, Azhar University, Egypt*
[c] *Department of Radiation Health, National Center for Radiation Research and Technology, Egypt*

Received 26 June 2012; accepted 20 August 2012
Available online 2 November 2012

KEYWORDS

Down syndrome;
Advanced glycation end products receptors;
CD34;
Nestin

Abstract On the trisomy Down syndrome Critical Region (DSCR1) is located the APP gene, which accelerates amyloid peptide protein (APP) expression leading to cerebral accumulation of APP-derived amyloid-beta peptides (Aβ) and age-dependent cognitive sequelae. Also DSCR1 attenuates endothelial cell proliferation and angiogenesis required for tissue repair. The aim of the present work is to determine markers of neural degeneration and regeneration in the blood of young and adolescent Down syndrome (DS) patients as well as controls. Markers of regeneration were measured in terms of circulating mononuclear cells expressing Nestin and CD34, while markers of degeneration were measured in terms of plasma $A\beta_{42}$ and advanced glycation end products receptors (RAGES). Results showed a significant increase in plasma $A\beta_{42}$ (20 ± 5.1 vs. 11.9 ± 3.4) and RAGES leucocytes mRNA relative expression (1.9 ± 0.2 vs. 1.1 ± 0.6) in adolescent DS patients compared to young DS. Both parameters were also significantly increased in DS compared to controls: $A\beta_{42}$ (15.4 ± 5.9 vs. 12. 3 ± 4.5); RAGES (1.4 ± 0.5 vs. 0.7 ± 0.2). Nestin (5.2 ± 1.4 vs. 6.3 ± 0.6) and CD34 (52 ± 2.5 vs. 53 ± 4.7) were non-significantly lower in adolescent DS patients compared to young DS, but significantly lower in DS patients compared to controls: Nestin (6.3 ± 1.5 vs. 9 ± 4.4); CD34 (54 ± 3.4 vs. 60 ± 4.8). The significant decrease in the number of mononuclear cells bearing Nestin and CD34 markers accompanied by a significant increase in $A\beta_{42}$ and RAGES indicate that degeneration in DS is an ongoing process, which is not counterbalanced by the regenerative mechanism.

* Corresponding author.
E-mail address: ekab@link.net (E. Abdel-Salam).

Peer review under responsibility of Ain Shams University.

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1. Introduction

DS is one of the most common and the best known of all malformation syndromes, and accounts for nearly 30% of moderate to severe mental retardation. Its estimated prevalence is one out of 800 live births [1]. The molecular basis for DS is

1110-8630
http://dx.doi.org/10.1016/j.ejmhg.2012.08.008

the triplication of chromosome 21, referred to as trisomy 21 (Ts21). Chromosome 21 is 45 Mb long and contains 303 genes [2]. Situated on this chromosome is the Down syndrome Critical Region (DSCR1) [3].

Located on the long arm of chromosome 21 is the APP gene that encodes the amyloid precursor protein, APP [4]. Endoproteolytic cleavage of APP yields the pathogenic amyloid-β peptides (Aβ) that progressively accumulate in the brain as diffuse and neuritic plaques, the critical factor contributing to the association of DS and Alzheimer disease (AD) [5,6]. Chromosome 21 triplication leads to increased dosage of the APP gene. Overexpression of APP in affected somatic cells accumulates Aβ in the brain, leading to age dependent cognitive sequelae [7,8] and reveals evidence of early-onset of AD neuropathology [9].

Recent studies have identified that the bone marrow contains a highly mobile population of mesenchymal cells that express mRNA for various markers of early tissue-committed stem cells (TCSCs), including neural TCSCs and endothelial TCSCs [10,11]. Neural TCSCs are mobilized into the peripheral blood following neural damage and chemo-attracted to the damaged neural tissue called circulating neural progenitor cells (NPCs). NPCs express surface markers identified as the CD34 and Nestin. Neural progenitor cells expressing Nestin and glial fibrillary acidic protein that are derived from the bone marrow, can integrate into the brain and generate microglia, neurons, and astrocytes and assist in neural regeneration [12,13].

Chromosome 21 distal segment is the site of the gene encoding the synthesis of superoxide dismutase enzyme (SOD). The excess of genetic information in patients with (DS) produces an increase in the catalytic activity of (SOD) as well as other gene products coded for on chromosome 21 [14]. Excessive SOD has been shown to increase the oxidative stress measured in terms of receptors advanced glycation end products (RAGES) mRNA expression and lipid peroxidation (MDA) [15].

It is well established that genetic condition such as Down syndrome causes premature aging accompanying global senescence in various organ levels and that the rate of aging in DS patients is nearly a twofold increase as compared to healthy subjects [16]. Aging is associated with a decline in immune responses [17], since it leads to an imbalance between degeneration and regeneration, which is the rate determining step in the development of the disease.

The aim of the present work is to determine neural markers of degeneration and regeneration in the blood of DS patients compared to controls, and compare such markers in young with adolescent DS. Markers of regeneration were measured in terms of cells expressing Nestin and CD34 in the blood of DS patients. Markers of degeneration were measured in terms of plasma $A\beta_{42}$ and RAGES.

2. Patients and methods

Patients were 40 Down syndrome cases. Age ranged from 2–15 years (mean 9.3 ± 4.1 year). Controls were 30 matched healthy normal children, whose age ranged from 2–14 years (mean 7.1 ± 4.2 years). DS patients were further sub-grouped into two age groups: below ten years (n = 23) from 2–8 years (mean 4.1 ± 1.3) and above 10 years (n = 17) from 10–15 years (mean12 + 1.3). DS patients were selected from cases already diagnosed by chromosomal karyotyping in the genetic unit, pediatric department, Cairo University. The selection criteria of the patients and controls were to be free from any infection and in good nutritive status. IQ test was carried out for DS patients and controls. Developmental milestones as age of sitting walking and the degree of hypotonia were recorded for both groups. Blood was drawn after an informed consent of the parents in accordance with the current revision of the 1975 Helsinki Declaration.

3. Biochemical Investigations

3.1. $A\beta_{42}$

This was carried out using Amyloid Beta (Aβ) ELISA kit (Millipore catalog number EZHS42 [18].

4. Reverse transcriptase-polymerase chain reaction (RT-PCR) for RAGE

Total RNA extracted from Histopaque 1077 (Sigma, Saint Louis MO, USA) separated lymphocytes using a QIAGEN RNA extraction kit (QIAGEN Inc, USA). The RNA samples were reverse transcribed using QIAGEN OneStep RT-PCR kit (QIAGEN Inc, USA), and processed according to the manufacturer's instructions. Aliquots (5 μl each) from the RT reaction were then used for PCR amplification with primer pairs for RAGE 5′-TGGAGACCATCAGTGCCTTTAT and antisense primer 5′-AGTTCATA-GTTGCCTGTCTGGG; glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which was used as an internal control, was amplified with the following primers: sense primer 5′-TGAAGG-TCGGAGTCAACG-GATTTGGT and antisense primers 5′-CATGTGGGCCA-TGAGGTCCACCAC. The polymerase chain reaction (PCR) was carried out in the following profiles: first cycle, 94 °C for 3 min, 58 °C for 1 min, and 72 °C for 1 min; second cycle to 30 cycles, 95 °C for 45 s, 58 °C for 40 s, and 72 °C for 45 s. The final cycle was 94 °C for 1 min and 72 °C for 10 min. The PCR reaction from RAGE receptors and GAPDH amplicons was separated by 1·5% agarose gels, stained with ethidium bromide and the gel image was evaluated under ultraviolet light and photographed for the appearance of specific bands: RAGE 535 bp and GAPDH, 292. Photos were scanned and quantification of each band was carried out using gene tools version 4. Each quantified data point was related to its individual GAPDH [16].

4.1. CD34. and Nestin quantification

To quantify EPCs in circulation, peripheral mononuclear cells were first isolated from the blood samples (0.5 mM EDTA). The isolated cells were labeled with the phycoenythrin (PE)-conjugated monoclonal nestin antibody and Fluorescein isothiocyanate (FITC) conjugated CD34 (Macs). The stained cells were washed with phosphate buffered saline and /BSA and then analyzed by flow cytometry at the Faculty of Medicine, Cairo University [19].

5. Results

Results showed a significant increase in plasma Aβ 42 (20 ± 5.1 vs. 11.9 ± 3.4) and RAGES leucocytes mRNA rela-

Table 1 Mean and SD of RAGES relative expression and amyloid beta peptide among Down syndrome patients below ten and above ten compared to each other and compared to controls.

	Controls	DS Patients	*P*	DS patients below 10 (n = 23)	DS patients above 10 (n = 17)	*P*
RAGES relative expression	0.7 ± 0.2	1.4 ± 0.5	$P < 0.0001$	1.1 ± 0.6	1.9 ± 0.2	$P < 0.0001$
Amyloid beta peptide 42	12. 3 ± 4.5	15.4 ± 5.9	$P < 0.01$	11.9 ± 3.4	20 ± 5.1	$P < 0.0001$

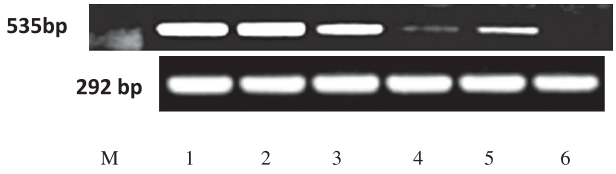

Figure 1 RAGE mRNA and GAPDH mRNA relative expressions among DS compared to controls. M denotes marker, lanes 1–3 represent DS patient, while 4–6 represent controls.

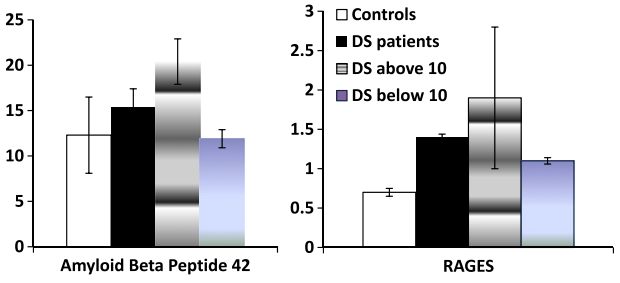

Figure 2 Mean and SD of RAGES relative expression and Aβ 42 among Down syndrome patients below ten and above ten compared to each other and compared to control.

tive expression (1.9 ± 0.2 vs. 1.1 ± 0.6) in adolescent DS patients compared to young DS. Both parameters were also significantly increased in DS compared to controls: (15.4 ± 5.9 vs. 12. 3 ± 4.5) for Aβ 42 and (1.4 ± 0.5 vs. 0.7 ± 0.2) for RAGES (Table 1, Fig 1 and 2). Nestin (5.2 ± 1.4 vs. 6.3 ± 0.6) and CD34 (52 ± 2.5 vs. 53 ± 4.7) were non-significantly lower in adolescent DS patients compared to young DS. The latter parameters were significantly lower among DS patients compared to controls: Nestin (9 ± 4.4 vs. 6.3 ± 1.5) and CD34 (60 ± 4.8 vs. 54 ± 3.4) (Table 2, Fig 3).

6. Discussion

The present study showed a significant increase in RAGES mRNA expression in DS patients compared to controls and was significantly higher among adolescent compared to young DS patients. This increase can be attributed to the increase of oxidative stress associated with DS patients. RAGE is a member of the immunoglobulin superfamily of cell surface molecules. It is a central signal-transduction receptor for AGES. Engagement of RAGE by these ligands activates key signal transduction pathways, leading to RAGE-mediated–enhanced expression of pro-inflammatory mediators that could be possible causes for neuro-degeneration associated with DS [20–22]. Studies carried out on the DS fetal brain cortex showed increased levels of glycation end products in comparison to controls, providing the

Table 2 Mean and SD of mononuclear cells expressing Nestin and CD34 among Down syndrome patients below ten and above ten compared to each other and compared to controls.

	Controls (n = 30)	DS patients (n = 40)	*P*	DS patients below 10 (n = 23)	DS patients above 10 (n = 17)	*P*
Nestin	9 ± 4.4	6.3 ± 1.5	$P < 0.0001$	6.3 ± 1.6	6.2 ± 1.4	$P > 0.05$
CD34	60 ± 4.8	54 ± 3.4	$P < 0.0001$	53 ± 4.7	52 ± 2.5	$P > 0.05$

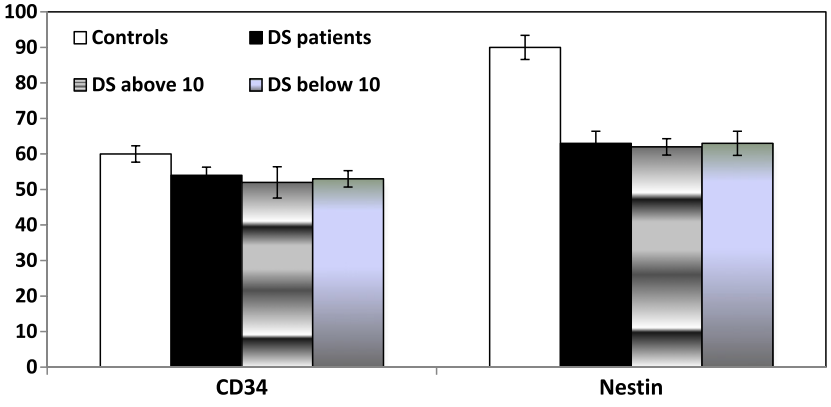

Figure 3 Mean and SD of mononuclear cells expressing CD34 and Nestin among Down syndrome patients below ten and above ten compared to each other and compared to controls.

evidence that accelerated brain glycoxidation occurs very early in the life of Down syndrome subjects [23].

The significant increase in $A\beta_{42}$ in DS compared to controls in the present study is due to the APP gene, located on the long arm of extra expressed chromosome 21 [5,7,24]. $A\beta_{42}$ progressively accumulates in the brain as neuritic plaques contributing to the association of dementia in DS and AD [25,26] and age-dependent cognitive sequelae [27,28]. The significant increase of $A\beta_{42}$ among adolescent DS compared to young DS gives an indication that $A\beta_{42}$ increases by aging in DS patients. Previous studies have shown increased plasma levels of $A\beta_{42}$ among adult DS either non-demented or with prevalent dementia [29,30]. Aβ deposits are found in brain capillaries, small arteries and arterioles producing cerebral amyloid angiopathy (CAA) [31,32] and causing disruption in the blood brain barrier [33], which has long been recognized as crucial for maintenance of the brain's micro-environment. [34].

CD34 was significantly lower among DS compared to controls while it was non-significantly decreased between adolescent and young DS patients. This finding gives clue to a previous *in vitro* data that showed that the vast majority of CD34 cells in the bone marrow of adult Ts65Dn mice (transgenic model of DS), exhibit a drastic reduction in their *in vitro* growth capacity. It showed also that in addition to neural antigens, cultured CD34 cells from trisomic and diploid mice also expressed mast cell markers [35] CD34 is an EPC/hematopoietic stem cell (HSC)-enriched population, which is capable of differentiation into cardiomyocytes *in vitro* [36] and into cardiomyocytes and smooth muscle cells *in vivo* [37].

Results of the present study showed that mean ± SD of mononuclear cell expressing Nestin surface marker was significantly lower among DS patients compared to control. These results are in agreement with a previous study [38], who found that Ts21 transgenic mice for DS have reduced numbers of neural progenitors [39]. Very little data are available describing neurogenesis in subjects with DS. It has been suggested that stem cells in the DS brain may be incapable of differentiating correctly during neurogenesis to form functional neuronal connections leading to the higher incidence of Alzheimer Disease in DS patients [39]. *In vitro* studies have shown that both hematopoietic and neural stem cells (NSC) from patients affected by DS show signs of accelerated aging [40]. Concurrently, DS brains weigh less, and the cerebral cortices have simpler convolutions than age-matched euploid brains [41,42]. A dramatic reduction in the number of neurons in the developing cerebral cortex of DS patients has been consistently reported [40,43].

In conclusion the significant decrease in the number of mononuclear cells bearing Nestin and CD34 markers, accompanied by a significant increase in amyloid β-peptide 42 and RAGES mRNA indicates that neural degeneration in DS is an ongoing process, which is not counterbalanced by the regenerative mechanism.

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