

## Serum S100B and intelligence in children with Down syndrome

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### Abstract

**Background** Down syndrome is characterized by physical and mental retardation and caused by chromosome 21 (Hsa21) abnormalities. The S100B is a protein that is overproduced in Down syndrome due to overexpression of chromosome 21 genes. Comorbidities caused by S100B in Down syndrome are cognitive deterioration and early onset of dementia.

**Objective** To assess for a possible association between S100B protein and intelligence levels in children with Down syndrome.

**Method** This cross-sectional study included students in a special needs school in Padang, West Sumatera, who had the characteristic clinical features of Down syndrome and trisomy 21 by chromosome analysis. Examination of S100B levels was carried out using an enzyme-linked immunosorbent assay (ELISA) method. Intelligence quotient (IQ) was measured using the 4th edition of the Wechsler Intelligence Scale for Children (WISC-IV) method.

**Results** A total of 39 children with Down syndrome participated in the study. There were 25 children with mild mental retardation and 15 children with moderate-severe mental retardation. The mean S100B levels were not significantly different between groups [479.1 (SD 204) pg/mL in the mild mental retardation group and 458.7 (SD 158) pg/mL in the moderate-severe mental retardation group;  $P > 0.05$ ]. The mean S100B level was significantly higher in subjects aged  $\leq 10$  years than in those aged  $> 10$  years [566.9 (SD 210.0) pg/mL and 434.4 (SD 167.2) pg/mL, respectively ( $P < 0.05$ )].

**Conclusion** There is no association between S100B and intelligence levels in children with Down syndrome. There is a significant association between higher S100B levels and younger age in children with Down syndrome. [Paediatr Indones. 2019;59:125-9; doi: <http://dx.doi.org/10.14238/pi59.3.2019.125-9>].

**Keywords:** Down syndrome; S100B; intelligence level

Children with Down syndrome have abnormal physical and mental development. Down syndrome is caused by failure of chromosome 21 (Hsa21) to separate during meiosis. This syndrome is characterized by mild-to-moderate mental retardation, craniofacial abnormalities, cardiovascular and gastrointestinal disorders, as well as immune deficiency.<sup>1-4</sup> Down syndrome is one of the most common congenital disorders in children, estimated to comprise 0.45% of human conceptions.<sup>5</sup> The incidence of trisomy Hsa21 worldwide was estimated to occur in 1 per 319-1,000 live births.<sup>6</sup> Idris *et al.* reported that 1,987 Down syndrome patients underwent chromosome analysis at the University of Indonesia from 1992-2004.<sup>7</sup> At Dr. M. Djamil Hospital in Padang, West Sumatera, 95 cases of Down syndrome were reported from 2009 to 2012, but only a small percentage had chromosomal examinations.<sup>8</sup> Chundrayetti noted that 39 children had Down syndrome in special needs schools in Padang in 2017, with chromosome examination results consistent with trisomy 21.<sup>8</sup>

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Down syndrome is the most common cause of intellectual disorders in children. Although the level of cognitive impairment varies widely, 80% of children with Down syndrome have mild-to-moderate intellectual disorders.<sup>1</sup> Overexpression of genes in the Down Syndrome Critical Region (DSCR) on the 21q22.1-21q22.3 segment is thought to be the cause of Down syndrome's clinical manifestations. Some genes in DSCR encode for proteins associated with neurocognitive disorders including amyloid precursor protein (APP), superoxide dismutase 1 (SOD1), dual-specificity tyrosine-phosphorylated and regulated kinase 1A (DYRK1), as well as S100B.<sup>9</sup>

The S100B protein is a member of the S100 protein family, with its gene located on chromosome 21q22.3. In normal conditions, S100B provides protective and neurotropic effects during brain development, the early stages of brain injury, and the regeneration process of injured peripheral nerves. In healthy individuals, S100B levels are at the highest concentration at early age of development, decreasing through adolescence and adulthood, then increasing with aging.<sup>9</sup> At high levels above the normal range, S100B has toxic effects on neurons through excessive production of reactive oxygen species (ROS), which eventually causes neuronal cell and astrocyte apoptosis.<sup>10</sup> The involvement of chromosome 21 in intellectual disorders in Down syndrome is thought to be related to S100B protein. Overexpression of genes on chromosome 21 which occurs in Down syndrome causes an increase of S100B protein levels, leading to neurotoxic effects on neuron cells and astrocytes.<sup>11</sup>

This study was aimed to assess for a possible association between S100B protein and intelligence levels in children with Down syndrome.

## Methods

This cross-sectional study was conducted at special needs schools in Padang, West Sumatera, and the Biomedical Laboratory of Andalas University Faculty of Medicine in February to March 2018. This study was approved by the Ethics Committee of the Universitas Andalas Medical School.

The study population comprised students from the special needs schools in Padang who met the inclusion criteria of clinical signs of Down syndrome

and trisomy 21 by chromosome analysis. Exclusion criteria were a history of brain infection, brain trauma, epilepsy, cerebral palsy, history of heart surgery, or schizophrenia. Serum S100B was measured using enzyme-linked immunosorbent assay (Elabscience Biotechnology Co. Ltd). The IQ level was measured using the Wechsler Intelligence Scale for Children (WISC-IV) 4<sup>th</sup> edition.<sup>12</sup> Data were processed using SPSS version 15 software. T-test was used to analyze for an association between serum S100B and intelligence levels. Results with P values <0.05 were considered to be statistically significant.

## Results

Demographic characteristics of the 39 children with Down syndrome who fulfilled the inclusion criteria are shown in **Table 1**. Most subjects were male and > 10 years of age. Most subjects' mothers were >35 years of age at the time of childbirth. There were more subjects with mild than moderate-severe mental retardation.

**Table 1.** Demographic characteristics of subjects

Characteristics	(N=39)
Sex, n	
Male	25
Female	14
Age, n	
≤ 10 years	11
> 10 years	28
Maternal age at childbirth, n	
< 35 years	10
≥ 35 years	29
Intelligence level, n	
Mild mental retardation	25
Moderate-severe mental retardation	14

The S100B levels in children with Down syndrome were normally distributed ( $P < 0.05$ ), with means of 479.1 (SD 204) pg/mL in the mild mental retardation group and 458.7 (SD 158) pg/mL in the moderate-severe mental retardation group. There was no statistically significant association between mean S100B levels and intelligence in children with Down syndrome ( $P = 0.749$ ) (**Table 2**).

Association analysis between serum S100B level and age is shown in **Table 3**. The mean S100B level was significantly higher in subjects aged ≤ 10 years

**Table 2.** Analysis of S100B and intelligence levels in children with Down syndrome

Age	S100B level, pg/mL		P value
	n	Mean (SD)	
Mild mental retardation	25	479.1 (204)	0.79*
Moderate-severe mental retardation	14	458.7 (158)	

\*T-test

**Table 3.** Association between S100B level and age in children with Down syndrome

Age	S100B level, pg/mL		P value
	n	Mean (SD)	
≤ 10 years	11	566.9 (210.0)	0.045*
>10 years	28	434.4 (167.2)	

\*T-test

than in those aged > 10 years [566.9 (SD 210.0) pg/mL and 434.4 (SD 167.2) pg/mL, respectively (P<0.05)].

## Discussion

The majority of study subjects were male, with a male: female ratio of 1.8:1. Johnson in 2006 also found more males with Down syndrome than females.<sup>13</sup> Most Down syndrome children (about 85%) have mild levels of mental retardation, whereas severe mental retardation occurs in 0.3-0.5% of the Down syndrome pediatric population.<sup>14</sup> In our study, mild mental retardation was more common (64.1%) than moderate-severe mental retardation. The incidence of Down syndrome increases in women who give birth at >35 years of age.<sup>7</sup> We also noted that 74.4% of our subjects' mothers were over 35 years of age at the time of childbirth.

Overexpression of S100B in neural progenitor cell (NPC) were isolated from frontal cortex of postmortem fetuses with Down syndrome causes an increase in the formation of reactive oxygen species (ROS) and activation of the stress response. Activation of this pathway results in compensatory aquaporin-4 expression. Aquaporin-4 expression can be induced by direct exposure to ROS, and an inhibition of aquaporin-4 by siRNA resulted in elevated levels of ROS following S100B exposure. Finally, increased levels of ROS induced by S100B and loss of expression

of aquaporin-4 led to increase neuronal cell death.<sup>15</sup> Some studies reported increased S100B levels in Down syndrome. Kato *et al.* conducted a study of S100B protein levels in individuals with Down syndrome aged 10-40 years in Japan. They noted higher S100B protein levels in those with Down syndrome compared to individuals without Down syndrome. This high S100B protein level was thought to be related to cognitive impairment in Down syndrome.<sup>16</sup> Similarly, Netto *et al.* showed increased S100B protein levels in 48 children with Down syndrome compared to controls, as well as a possible relationship between S100B protein levels and neurodegenerative lesions that occur in Down syndrome.<sup>9</sup> Previously, Netto *et al.* had reported higher levels of S100B in amniotic fluid of mothers carrying fetuses with Down syndrome compared to that of mothers carrying normal fetuses.<sup>17</sup>

In our study, the mean S100B level was 479.1 (SD 204) pg/mL in the mild mental retardation group and 458.7 (SD 158) pg/mL in the moderate-severe mental retardation group. Similar to previous studies, the mean S100B level of children with Down syndrome in our study was higher than the normal S100B cut-off value of 20-150 pg/mL. Boussard *et al.* noted that S100B protein concentration above the cut-off value of 150 pg/mL was considered pathological.<sup>18</sup> S100B protein is produced mainly by astrocytes in the brain, with increasing levels consistently indicative of a neuropathological process. The main advantage of measuring S100B in serum is that increases in serum can be easily measured, providing a sensitive method for detecting central nervous system dysfunction. Although S100B is also produced by other extracranial cells, in previous studies extracranial S100B did not affect serum S100B level.<sup>19</sup>

Many studies support an important role of S100B in central nervous system development.<sup>18,20-24</sup> Extracellular S100B at nanomolar concentrations acts as a potent neurotropic and gliotropic agent. The effects of S100B on cognitive function include increased cell function, suppression of neurovascular inflammation, as well as increased conduction and transmission of nerve impulses.<sup>21</sup> S100B is a potential marker of trauma, infection, or pathological disorders in the central nervous system. In this context, S100B is described as an acute-phase response protein. Increased S100B level in response to

various stressors can lead to pathological symptoms of cognitive impairment. The S100B expression also increases selectively in astrocytes in the aging process, Alzheimer's disease, and in individuals with chromosome 21q22.3 excess. This finding was seen in the pathophysiology of neurodegenerative disorders that are typical of Alzheimer's disease and Down syndrome.<sup>22</sup> Several studies have shown associations between S100B level and cognitive impairment in several disease conditions. Pedersen *et al.* found a significant association between S100B levels and memory disorders in schizophrenic patients.<sup>23</sup> Zhai *et al.* also found a significant association between S100B gene polymorphism and increased level of S100B protein with visuospatial disability in schizophrenic patients.<sup>24</sup> Furthermore, Li *et al.* reported increased S100B levels in adult patients with post-operative cognitive dysfunction (POCD).<sup>25</sup>

Azmitia *et al.* examined the effect of S100B overexpression on the behavior and morphology of neurons in mouse models. Their study showed that S100B excess caused cognitive deficits, obstacles to adapting, and decreased response to danger. In elderly mice, markers of apoptosis increased and signs of neuroinflammation occurred. In the end, transgenic S100B mice showed neurodegeneration and hyperphosphorylation of the structure of Tau, as seen in the late stages of Down syndrome and Alzheimer's disease.<sup>11</sup> However, in our study, there was no significant association between S100B levels and intelligence in children with Down syndrome.

In normal brain conditions, S100B is generally at the highest concentration at the beginning of development, decreases in adolescence and adulthood, and increases again with aging. Netto *et al.* found no correlation between S100B levels and age in children with Down syndrome, as found in normal children,<sup>9</sup> but in our study, there was a significant association between higher S100B levels and younger age. This different result may have been due to our differing age distribution.

This study had several limitations. The sample size was considered small for assessing for a possible association between variables. We also did not use a control group, so we could not compare to S100B levels in healthy individuals.

In conclusion, most children with Down syndrome have mild mental retardation and S100B levels above the normal range. However, there is no

association between S100B levels and intelligence level in children with Down syndrome. There is an association between serum S100B levels in Down syndrome patients with age, as it is in normal individuals. Further studies with a larger sample size and control group should be conducted to confirm these findings.

## Conflict of interest

None declared.

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