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Sara Bakhsha^{§*}, Mohamed A. Elmisiry[§], Nehad Nasef[#], Mohamed E. Abdraboh[§]

[§] Zoology dept., faculty of Science, Mansoura University, Egypt.

[#] Pediatric dept., faculty of Medicine, Mansoura University, Egypt.

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Original Article

Role of endogenous melatonin downregulation in Young children Down syndrome patients' on liver and thyroid gland functions

Sara Bakhsha^{§*}, Mohamed A. Elmisiry[§], Nehad Nasef[#], Mohamed E. Abdraboh[§]

[§] Zoology dept., Faculty of Science, Mansoura University, Egypt.

[#] Pediatric dept., Faculty of Medicine, Mansoura University, Egypt.

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Abstract

Melatonin is a pineal gland hormone that has a potent antioxidant characteristic. The presence of an extra copy of SOD on chromosome 21 in Down syndrome (DS) patients' was correlated with free radicals generations that affect several internal organs. This study aims to correlate the effect of DS dependent oxidative stress on melatonin expression and liver and thyroid gland functions. Blood plasma of twenty young children DS patients' and ten healthy controls were collected and tested for the levels of SOD, H₂O₂, melatonin, Liver functions enzymes, total proteins, TSH, T3 and T4 using colorimetric and ELISA techniques. SOD overexpression leads to H₂O₂ accumulation and melatonin downregulation which in turn leads to a status of oxidative stress affecting Liver functions indicated by total protein significant downregulation. A direct impact of oxidative stress and melatonin downregulation was correlated to DS -dependent hypothyroidism of significant TSH upregulation and T3 and T4 downregulation. In conclusion, the reduction at melatonin endogenous levels in plasma of young children DS patients' may highlight its role in upsurge the DS-dependent oxidative stress invulnerable pathological features. This study recommends the implication of melatonin in prospective clinical trials of DS therapy.

1. Introduction

Down syndrome (DS) is one of the most prevalent genetic disorder affecting 1 in 1500 babies worldwide. It also, known as trisomy 21 due to trisomy of whole or part of chromosome 21 (Moses *et al.*, 2017). The abnormality of chromosome 21 in DS appeared during meiotic cellular division phase. DS is a maternal age dependent factor where, the incidence of DS babies increases with the increase of the mother age, it may also appeared with younger women (Allen *et al.*, 2009). DS is characterized by a number of phenotypes that display less penetrance, including cardiovascular, immunological, skeletal and motor alterations (Das and Reeves, 2011). DS patients' are characterized by intellectual disability and millions of DS patient have various health issues including learning and memory dysfunction, congenital heart diseases, Alzheimer's

diseases (AD), leukemia, cancers and Hirschprung disease (Asim *et al.*, 2015). The most prominent features of DS are intellectual disability and the early appearance of Alzheimer's disease (AD)-like neuropathology that affects 100 % of individuals with this condition (Wiseman *et al.*, 2015). There are three different types of Down syndrome: trisomy 21 or nondisjunction (95%), Robertsonian translocation (4%) and mosaicism (1%). The sequencing of Hsa 21 proved to be an important factor in the progression of DS research and led to further insight into genotype-phenotype correlations associated with DS (Dierssen, 2012). A number of studies have provided sufficient evidences that the DS phenotype is associated with oxidative stress, mainly due to SOD-1 overexpression that has been investigated in number of *in vitro*, *in vivo* studies (Zigman, 2013). A number of oxidative

stress parameters were measured in brains, blood cells and body fluids from DS patients, pointing out the significant increases in oxidative DNA damage, lipid peroxidation, plasma levels of uric acid and allantoin, along with lower-than-normal levels of xanthine and hypoxanthine (Perluigi *et al.*, 2012). Early studies reported deficiencies in certain mitochondrial enzymes, including monoamine oxidase, cytochrome oxidase and isocitrate dehydrogenase from blood platelets of DS patients, similar to defects in cytochrome oxidase (Complex IV) found in blood platelets and in brain tissue from patients with AD (Pallardó *et al.*, 2010). Under physiological conditions, cell apoptosis plays a central role in establishing the number of and connections between neurons during development. One mechanism proposed to be the neurobiological correlate of cognitive disability in DS individuals is the widespread hypo-cellularity found in their brains, which can lead to altered synaptogenesis, connectivity and synaptic plasticity.

Melatonin (Nacetyl-5-methoxytryptamin) can be synthesized from tryptophan via the methoxyindole pathway, which is mainly pineal gland dependent (Oliveira *et al.*, 2018). The rhythm of melatonin secretion is characterized by the existence of a peak of pineal secretion during the night, while plasma melatonin levels fall during the day (Reiter *et al.*, 2016). These levels measured during daylight hours are assumed to be of extra pineal secretion. Melatonin and its metabolites may increase the enzyme activity of Superoxide dismutase may be of particular importance for patients with DS, among whom the levels of Superoxide dismutase are already above normal, due to the effect of the gene load (Izzo *et al.*, 2018). This study aimed to investigate the relation between melatonin secretion and modulation of TSH expression in DS patients' which appears mainly as a function of oxidative stress.

2. Materials and Methods

Patients' selection and exclusion criteria:

This study was carried out in agreement with the Declaration of Helsinki and Spanish laws regarding data privacy and approved by Clinical research ethical committee of the Mansoura University (DZ18005). Individuals were recruited in the children hospital at Mansoura University where the study took place. Only those whose measurements regarding the variables of the present report were available at all time points (n=20) were involved in this study. Young children DS patients' with gall bladder problems were excluded. Upon arrival at the children hospital, and prior to participating in the trial, the legal guardians were informed about the protocol and gave their written informed consent.

Sample Collection:

The blood samples of the study groups were randomized into two groups 1) DS young children patients' (n=20). 2) Normal controls of healthy young children presented at children hospital for

phlebotomy or day case procedures (n=10). All participants were with an age range from 12 to 18 months at the beginning of the study. 2 ml blood samples were taken from antecubital vein to collection tubes with EDTA throughout the study (6 months) a month after month. Samples were then centrifuged at 1500 g for 10 min and the plasma was separated and kept frozen at -80°C until used.

Determination of melatonin concentration in plasma

The collected patients' plasma were assessed for the level of endogenous melatonin using Creative Diagnostics ELISA detection kits according to manufactures' instructions (Creative Diagnostics, USA).

Determination of antioxidant enzymes activities in plasma

Activity of SOD, CAT and GPx enzymes was measured in patients' plasma using biodiagnostic colorimetric detection kits (Biodiagnostics, Giza). H₂O₂ and malondialdehyde (MDA) levels were measured in patients' plasma as well using colorimetric detection kits according to manufactures' protocol (Biodiagnostics, Giza).

Determination of thyroid hormones levels and liver function in plasma

The plasma of the DS young Children patients' that were on medication with or without Eltroxin for hypothyroidism were assessed for levels of Thyroid Stimulating hormone (TSH), Triiodothyronine (T3) and Thyroxine (T4) using automated immunoassay analyzer (IMMULITE 2000, Siemens Corp., NY). The levels of TSH, T3 and T4 in plasma of healthy children was assessed as a reference of normal controls. The level of total protein and the levels of liver enzymes AST and ALT was assessed using Spinreact kit (Spinreact, Spain) according to manufacturer's protocol.

Statistical Analyses: The analysis was carried out with the use of GraphPad Prism 5.03 software. Continuous data were tested for normality of distribution prior to any calculations. All continuous data were normally distributed and were presented in mean \pm SD. Statistical significance was set at $p < 0.05$.

3. Results

Effect of DS on expression of Melatonin and SOD

The pre-diagnosed DS young children patients' at the children hospital, Mansoura University were first tested for the plasma level of endogenous melatonin. The data revealed a significant diminishment at the mel. levels in plasma of children at the first month and even after 3 and 6 months of the study compared to normal controls (fig 1). Accordingly, the expression of SOD was spotted out through the three time points of the study (1, 3 & 6 months) in young children DS patients' and the data revealed a significant upregulation at SOD levels compared to normal controls (fig. 1).

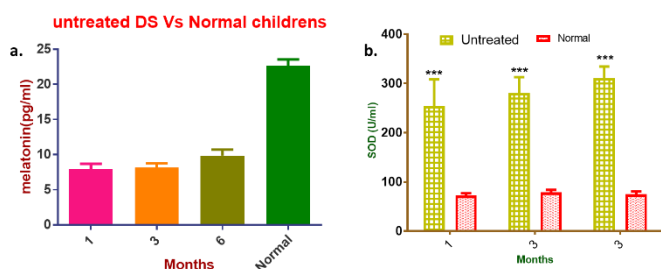


Figure 1. Effect of DS on the expression levels of melatonin and SOD. (a.) Significant downregulation at melatonin expression levels were shown in plasma of young children DS patients' compared to normal controls. (b.) A significant enormous upregulation at the expression of SOD enzyme was recorded in young children DS patients' compared to normal controls.

Effect of DS -dependent melatonin depression and SOD upregulation on Oxidative stress

The effect of DS on oxidative stress status was indicated by the significant time dependent upregulation at H₂O₂ generation levels compared to normal controls. Meanwhile, the data of the antioxidant enzymes CAT and GPx revealed a time dependent significant downregulation at their expression (fig.2). The effect of DS on upregulating the level of oxidative stress was finally illustrated by the significant time dependent upraise at lipid peroxidation marker MDA (fig.2).

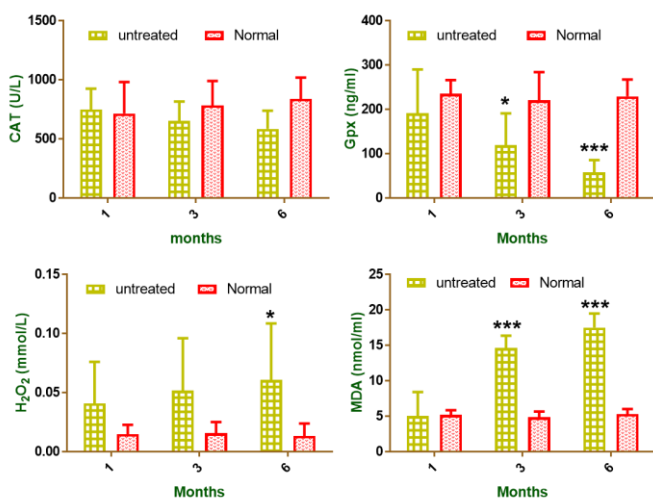


Figure 2. Effect of DS on the oxidative stress in Young children DS patients'. Assessment of H₂O₂ at 1,3 and 6 months of starting the study showed a significant upregulation at H₂O₂ generation levels were shown in plasma of young children DS patients' compared to normal controls. The expression of CAT and Gpx were significantly downregulated over the study duration in a time dependent manner compared to normal controls. The levels of MDA as a marker for oxidative stress indicated a time related development at lipid peroxidation in DS young children patients' compared to normal controls.

A significant time dependent increase at total protein levels that been recorded among DS patients' compared to normal controls. On the other hand, The screening for the effect of melatonin downregulation and oxidative stress upregulation on liver function enzymes AST and ALT among DS patients' revealed

a non-significant effect at their expression levels. Meanwhile, a significant time dependent decrease at total protein levels was recorded (fig. 3).

Effect of melatonin and oxidative stress on thyroid gland functions

A significant time dependent upraise at the levels of TSH were revealed among DS Eltroxin untreated patients'. Eltroxin significantly reduced the expression of TSH in a time dependent manner (fig.4). Furthermore, the expressions of the thyroid gland hormones T3 and T4 were significantly downregulated in DS young children patients' compared to normal controls. This effect was significantly over countered by Eltroxin in a time dependent manner (fig. 4).

4. Discussion

The antioxidant characteristics of melatonin illustrated its cytoprotective effects in protecting cells from oxidative stress related diseases such as osteoarthritis, cardiovascular diseases and cancer (Tomás-Zapico and Coto-Montes, 2007).

The data revealed a significant decrease at Melatonin expression levels in DS young children patients compared to their reference levels in healthy children. A significant downregulation at the expression of serotonin, Melatonin and its metabolite kynurenic acid was recorded during day time (periods of light) in DS children compared to healthy controls (Uberos et al., 2010).

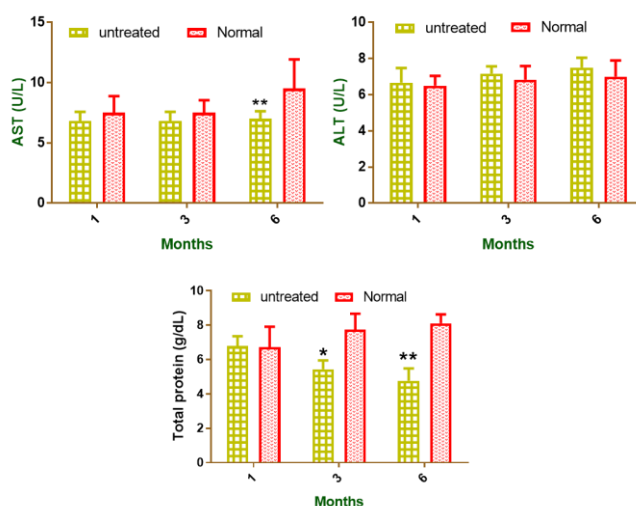


Figure 3. Effect of DS dependent oxidative stress on liver functions of Young children DS patients'. The data revealed a non-significant effect of oxidative stress on liver enzymes. A significant reduction at total protein levels were shown in plasma of young children DS patients' compared to normal controls in a time dependent manner during the length of the study.

Alteration at the endogenous Mel expression profile has been recorded in several genetic disorders such as DS, Prader-Willi syndrome and autism spectrum disorder (ASD) (Schwichtenberg and Malow, 2015). The documented low activity of the acetylserotonin O-methyltransferase (ASMT), the last enzyme implicated in the melatonin synthesis

pathway, and the newly identified mutations in the ASMT gene in patients with ASD may partly act in explaining the downregulation at Mel. levels in genetic diseases (Jonsson *et al.*, 2010; Etain *et al.*, 2012).

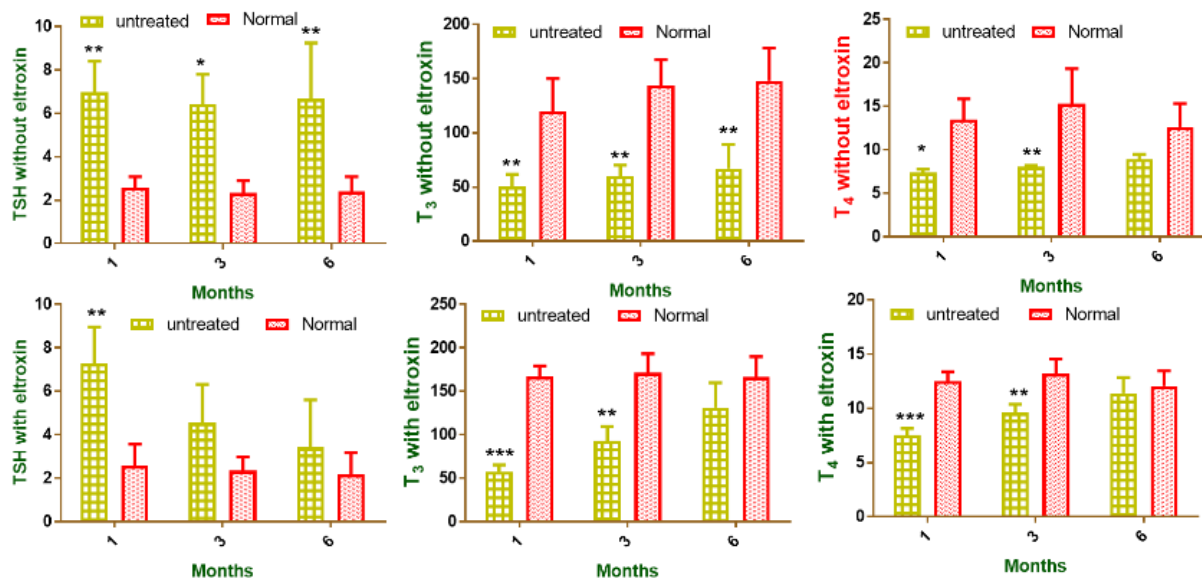


Figure 4. Effect of oxidative stress on DS-related hypothyroidism of Young children DS patients’. The data revealed a significant upregulation at TSH expression and downregulation of thyroid gland enzymes T3 and T4 throughout the study. This effect was counteracted by Eitroxin medication in a time dependent manner.

Meanwhile, the significant increase at SOD expression compared led to a significant upregulation at H₂O₂ expression which is a typical pattern of expression for both of them in DS patients’ in comparison with healthy children. The role of melatonin in scavenging H₂O₂ and enhancing antioxidant enzyme activity was elucidated by several literatures (Sies, 2015).

This increase at H₂O₂ levels led to an imbalance at the activity of the antioxidant enzymes CAT and Gpx and a subsequent significant increase at the level of lipid peroxidation marker MDA as an indication of oxidative stress, in a time dependent manner.

Several studies elucidated the upraise of oxidative stress markers in DS Patients’ as they correlated the typical DS characteristics of high levels of SOD and H₂O₂ in blood plasma and an oxidative stress upraise due to the imbalance of antioxidant enzymes capacity (Rodriguez *et al.*, 2004; Campos and Casado, 2015; Perondi *et al.*, 2018).

This upregulation at oxidative stress status would affect the overall body health which was indicated by the significant downregulation at total proteins. Interestingly, non-significant effect at liver enzymes AST and ALT was indicated in DS young children patients’ compared to healthy children, Recent studies illustrated the protective effect of melatonin against chemical and alcohol abuse induced liver damage (Zhang *et al.*, 2017).

On the other hand, the data revealed a significant effect of DS-correlated downregulation of melatonin on induction of hypothyroidism in young children’s patients’ by modulating the TSH, T3 and T4 expressions profile compared to healthy controls. A fascinating effect of Eitroxin was recorded in significantly restoring the normal activity of thyroid hormones in a time dependent manner compared to healthy controls.

The correlation between DS disorder and hypothyroidism has been elucidated in a recent literature (Amr, 2018). A clinical study, conducted on twenty women, indicated a significant positive correlation between TSH levels and melatonin expression in hypothyroidism and a negative correlation with TSH in hyperthyroidism (Soszyński *et al.*, 1988; Lewinski, 2005).

In conclusion, this study sheds the light on the effects of endogenous mel. deprivation in DS young children patients’ on amplifying the invulnerable effects of DS-dependent oxidative stress on the liver functions and DS related hypothyroidism. This research considered as a founding ground for prospective application of mel. in clinical trial of DS therapy at early stages of development.

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