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MOLECULAR MECHANISMS OF TOCOTRIENOL-RICH FRACTION AND VITAMIN C IN AMELIORATING OXIDATIVE STRESS IN DOWN SYNDROME

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ABSTRACT

Down syndrome (DS) is a common chromosomal abnormality occurring in about 1 in 700 live births. The gene encoding for Cu-Zn superoxide dismutase (SOD) is present on chromosome 21, thus, individuals with triplicates of this chromosome are postulated to be in a state of oxidative stress. Previously, it was shown that DS was associated with oxidative stress and impairment of immune function. It is postulated that the supplementation with tocotrienol rich fraction (TRF) (150 mg) and vitamin C (500 mg) will relieve DS subjects from oxidative stress i.e the improvement of the DNA damage levels, antioxidant-oxidative stress status, plasma levels of vitamin C and E and miRNA levels. This study aimed to examine the molecular mechanism of TRF and vitamin C to combat oxidative stress in DS. This was a cross-sectional study carried out with informed consent on DS subjects from several Pusat Pemulihan Dalam Komuniti (PDK) in Klang Valley after ethical approval was obtained. The demographic data of the study participants were gathered through questionnaires that were distributed to parents. Sixty DS subjects (2 to 29 years old) were recruited and randomly assigned to receive either supplementation (n=30) with TRF (150 mg) and vitamin C (500 mg) or placebo (n=30), daily for 6 months. Blood samples were obtained from each subject at 0, 3rd and 6th months for the analysis. Antioxidant-oxidative stress status was assessed from the measurement of antioxidant enzyme activities in erythrocytes, viz, SOD, catalase (CAT) and glutathione peroxidase (GPx). DNA damage was measured using comet assay and 8-hydroxy-2'-deoxyguanosine (8-OHdG) level was evaluated using ELISA to measure the endogenous oxidative damage to DNA. The 8-isoprostane level was measured to study the biomarker of oxidative stress. Vitamin C and E levels were assessed using HPLC and miRNA analysis was performed using RT-PCR. This study showed the activities of antioxidant enzymes of DS subjects after supplementation with TRF and vitamin C were similar when comparing the baseline to 3 and 6 months ($p>0.05$). There were no associations between SOD, CAT and GPx activities and plasma level of vitamin C and E in treatment and control groups ($p>0.05$). There were no significant differences in the comet assay in treatment and control groups at 0, 3 and 6 months of supplementation ($p>0.05$). Increased plasma level of total vitamin E concentrations was observed with the duration of treatment, after 3 months (9.45±0.58 g/mol, $p<0.05$) and 6 months (10.15±0.72 g/mol, $p<0.05$), respectively in the treatment group and similar results were observed in plasma tocopherol and tocotrienol levels. Plasma vitamin C concentrations were not statistically different in treatment and control groups and also with the duration of treatment. The expression of miRNA; hsa-let-7c, hsa-miR-99a, hsa-miR-155, hsa-miR-125b and hsa-miR-520d showed no significant differences ($p>0.05$) in the expression compared to baseline at 0 month. A significantly upregulated expression of hsa-miR-99a (2.40±0.08 folds, $p=0.044$) and hsa-miR-802a (3.60±0.041 folds, $p=0.022$) were seen compared to controls after 3 months of intervention. A significantly upregulated expression of hsa-miR-99a (2.58±0.04 folds, $p=0.049$) and downregulated expression of hsa-miR-125b (3.51±0.01 folds, $p=0.049$) were found compared to controls at 6 months of intervention. In conclusion, daily supplementation with TRF (150 mg) and vitamin C (500 mg) for 6 months did not improve the oxidative stress level in DS subjects. Factors such as the duration of supplementation and the number of subjects should be addressed in future studies. Other factors such as genetic, epigenetic, and environmental may play a role in how the DS phenotype specifically that related to oxidative stress expresses itself in each individual.

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CHAPTER ONE
INTRODUCTION

1.1 Research Background

Down syndrome (DS) was first described around 155 years ago by John Landon Down and it is the most common genetic form of intellectual disability occurring in 1 to every 600–700 live births. However, other possible factors which may influence the prevalence of DS viz spontaneous abortion and miscarriage cases which mostly occur in the first trimester were not included in the statistical data (Jaruratanasirikul et al., 2017). A consistent correlation between prenatal diagnosis and birthrate contribute to the lower prevalence of Down syndrome (Huete-Garcia & Otaola-Barranquero, 2021).

An extra copy of chromosome 21, trisomy 21 causes DS and around 200-300 genes on chromosome 21 have been identified as the contributors to clinical features in these individuals. Trisomy 21 may occur by nondisjunction, translocation or mosaicism. Mosaicism is usually associated with fewer clinical features of DS (Bull, 2020). There is no specific factor that may contribute to the maternal risk with DS. However, the incidence of DS increases with the maternal age of the mother (Akhtar & Bokhari, 2020). Individuals with Down syndrome are at risk of other medical conditions such as congenital heart defect, leukaemia, anaemia and also high risk of developing Alzheimer's disease which is also associated with dementia (Krinsky-McHale et al., 2020). The health issues among DS individuals are complex and are associated with many other factors.

Overexpression of superoxide dismutase 1 (SOD1) which is located on chromosome 21 is believed to underlie the DS phenotype, leading to increased oxidative stress levels (Cowley Patrick et al. 2017). Oxidative stress is an imbalance in the pro-oxidants-antioxidants status with increased production of the former, this imbalance is the underlying factor in the development of DS-related pathologies. Superoxide dismutase (SOD) converts superoxide anion radicals to hydrogen peroxide which is then reduced to water by selenium-dependent glutathione peroxidase and catalase (Younus, 2018). Biomarkers of oxidative stress are elevated in DS individuals as previously described by many studies (Garlet et al., 2013; Cenvertini et al., 2016; Ferrari & Stagi, 2021). Among the parameters that were measured as indicators of oxidative stress