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Original Research Paper

Down Syndrome: Association of Oxidative Stress and Thyroid Function With Haematologic Profile

Medical Science

KEYWORDS : Down syndrome(DS),Malondialdehyde(MDA),Catalase(CAT), Glutathione peroxidase(GPX),Uric acid (UA),Superoxide dismutase(SOD).

*** DR.RESHMI.R**	ASSISTANT PROFESSOR,DEPARTMENT OF PHYSIOLOGY,GOVERNMENT TD MEDICAL COLLEGE,VANDANAM,ALAPPUZHA,KERALA-688005 *CORRESPONDING AUTHOR
DR.VIJAYALEKSHMI M.T	PROFESSOR AND HEAD, DEPARTMENT OF PHYSIOLOGY, GOVERNMENT TD MEDICAL COLLEGE, VANDANAM ,ALAPPUZHA,KERALA-688005
DR.DINESH ROY .D	CEO & SENIOR CYTOGENETICIST,GENETIKA, CENTRE FOR ADVANCED GENETIC STUDIES, TRIVANDRUM, KERALA – 695024
DR.GEETHA DAMODARAN	PROFESSOR, DEPARTMENT OF BIOCHEMISTRY, GOVERNMENT MEDICAL COLLEGE, KUMMANNUR, KONNI,KERALA-689691

ABSTRACT

Background:- Down syndrome (DS) or Trisomy 21 is the most common genetic cause of mental retardation . The phenotypic abnormalities and the morbidity in DS are due to higher gene dosage of chromosome 21, increased oxidative stress and thyroid dysfunction . Aims:- The association of oxidative stress and thyroid function with the haematological profile in DS was assessed.Methods and Material:-25 cases of Down syndrome was selected for the study after confirmation by karyotyping.6ml of blood was taken and send for haematological analysis and serum analysis of Malondialdehyde (MDA),Catalase ,Glutathione peroxidase, Uric acid, and Thyroid function. Statistical analysis used:-The mean and standard deviation of each of the variables were calculated . The association between the variables was calculated using Pearsons correlation. Results :DS cases showed significant increase of MCV ,significant decrease of WBC count, RBC count, Platelet count ,MCHC and neutropenia . The haematological variables showed a significant association with oxidative stress parameters . Conclusion :The haematologic profile in DS showed significant macrocytosis , anemia, leucopenia , neutropenia and thrombocytopenia.The increased oxidant stress in DS of this study, significantly altered the haematology of DS . Hence measures to decrease the oxidative stress may have a role in correcting the altered haematology profile in DS .

INTRODUCTION

Down syndrome(DS) or Trisomy 21 occurs in about 1:800 live births1 .The amplified gene products of chromosome 21 includes cystathionine beta synthase2 and cytoplasmic Cu-Zn-superoxide dismutase(SOD)3 among 300 other genes . The SOD overactivity leads to oxidative stress affecting every system in DS by damaging DNA ,lipids and proteins4 .The haematological abnormalities in this progeroid , prooxidant state of DS are neutropenia, thrombocytopenia,macrocytosis,transient abnormal myelopoiesis,myelodysplasia and myeloid leukemia 5. This case control study was done to assess the association of haematological profile with oxidative stress and thyroid status .

SUBJECTS AND METHODS

Karyotypically confirmed 25 cases of Down syndrome were selected for the study after getting informed consent from the parents.25 age and sex matched controls were selected from the neighbourhood. 6 ml of blood was taken from the cases and controls. . Ethical clearance was obtained from Institutional ethics committee (No.B6/14038/2011/EC66/2014).

Karyotyping was done by peripheral blood lymphocyte micro culture as described by Moorhead et al6, GTG banding as described by Seabright7 and chromosome identification according to International System for Human Cytogenetic Nomenclature.

Haematological parameters were measured using automated analyser. Malondialdehyde estimation (MDA) was done by Valipasha and Sadasivudu method 8 . Catalase activity was measured by the Hugo Aebi method 9 . Glutathione peroxidase estimation was measured by the decrease in absorbance at 340 nm on mixing the blood sample with Drabkins reagent and cumene hydroperoxide .Quantitative determination of uric acid was done using Trinder reaction10 . Thyroid function was assessed using ELISA.

Statistical analysis was done using the SPSS Software 17 . The results were expressed as mean + Standard deviation.p values < 0.05 was considered significant . Pearson correlation coefficient (r) was used to test the correlation of different variables.

RESULTS

Table1: Haematological Profile of Down syndrome and controls

Variable	Controls/ cases	Mean	SD	t	p value
Hb g/dl	DS	13.4	1.6	-0.305	0.762
	Control	13.5	1.1		
MCV (fl)	DS	96.8	6.9	12.169	0.000
	Control	79.2	2.3		
RBC (× 106 /µl)	DS	4.7	0.5	-2.753	0.008
	Control	5.0	0.3		
MCHC g/dl	DS	29.8	2.2	-7.046	0.000
	Control	33.8	1.8		
RDW (%)	DS	12.7	0.7	-3.826	0.000
	Control	13.5	0.9		
WBC (× 103 /µl)	DS	2.9	1.2	-13.583	0.000
	Control	6.8	0.9		
Neutrophil (%)	DS	17.4	7.2	-14.503	0.000
	Control	47.4	7.5		
Lymphocyte count (× 103 /µl)	DS	2.4	1.0	-3.807	0.000
	Control	3.3	0.6		
Platelet count (× 103 /µl)	DS	142.2	50.7	-4.818	0.000
	Control	204	39.2		

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It is evident from Table 1 that Down syndrome cases have significant macrocytosis,anemia, leucopenia, lymphocytosis,neutropenia and thrombocytopenia.

Table 2: Values of Oxidative stress parameters , Antioxidant enzymes and Thyroid status in DS and controls

Variable Mean ±SD	MDA (nmol/L)	GPX (u/l)	Catalase (Kat/gm Hb)	Uric acid (mg/dl)	TSH µIU/ml	F T3 pmol/L	F T4 pmol/L
DS	120.0 ± 28.6	945.4 ± 324.1	102.9 ± 22.1	5.7 ± 1.5	3.6 ± 4.2	8.0 ± 2.2	11.4 ± 2.1
Controls	60.2 ± 6.6	5501.0 ± 1145.9	77.4 ± 0.6	3.3 ± 0.7	3.9 ± 2.6	5.5 ± 2.6	19.2 ± 4.2

Table 3: Correlation of Hematological parameters with parameters of oxidant stress and antioxidant activity.p<0.05 considered significant.

Pearson Correlation	MDA (nmol/l)		GPX (u/l)		CAT (Kat/gmHb)		UA (mg/dl)	
Variables	r	p	r	p	r	p	r	p
MCV(fl)	0.720	0.000	-0.823	0.000	0.499	0.000	0.713	0.000
RBC count (× 106 /µl)	-0.345	0.014	0.361	0.010	-0.105	0.468	-0.088	0.543
MCHC(g/dl)	-0.633	0.000	0.767	0.000	-0.577	0.000	-0.456	0.001
RDW(%)	-0.339	0.016	0.469	0.001	-0.490	0.000	-0.384	0.006
WBC count (× 103 /µl)	-0.779	0.000	0.833	0.000	-0.595	0.000	-0.662	0.000
Neutrophil %	-0.782	0.000	0.858	0.000	-0.620	0.000	-0.714	0.000
Lymphocyte count (× 103 /µl)	-0.483	0.000	0.472	0.001	-0.333	0.018	-0.388	0.005
Platelet count (× 103 /µl)	-0.356	0.011	0.610	0.000	-0.453	0.001	-0.589	0.000

Results in Table 2and Table 3 shows the following findings in Down syndrome. The WBC count has a significant negative correlation with MDA , GPX,CAT and UA values. RBC count has significant correlation with MDA,GPX and CAT values. MCV has a significant correlation with MDA,GPX,CAT and UA levels.Platelet count shows significant negative correlation with MDA ,UA ,CAT values and positive correlation with GPX values. Neutropenia shows significant negative correlation with MDA values,CAT and UA values and a significant positive correlation with GPX values.

Table 4: Correlation of Hematological parameters with thyroid status.

Pearson Correlation	FT3(pmol/l)		FT4(pmol/l)		TSH(µIU/ml)	
Variables	r	p	r	p	r	p
WBC count(× 103 /µl)	-0.548	0.000	0.673	0.000	-0.041	0.779
RBC count(× 106 /µl)	-0.183	0.203	0.265	0.063	0.281	0.056
MCV(fl)	0.472	0.001	-0.684	0.000	-0.105	0.466
MCHC (g/dl)	-0.442	0.001	0.496	0.000	-0.013	0.930
Platelet count(× 103 /µl)	-0.327	0.020	0.556	0.000	-0.117	0.420
Lymphocyte %	0.423	0.002	-0.723	0.000	-0.004	0.977
Neutrophil %	-0.418	0.003	0.751	0.000	0.017	0.909
Lymphocyte count(× 103 /µl)	-0.264	0.064	0.394	0.005	-0.055	0.706
RDW%	-0.188	0.191	0.450	0.001	-0.384	0.006

Results in Table 4 shows that the haematological variables correlated significantly with FT3 and FT4 values, but failed to show any association with TSH values.

DISCUSSION.

In the present case control study, DS cases shows significant macrocytosis . The macrocytosis and reduced blood cell survival in DS has been documented in previous studies11-14. The correlation of macrocytosis with oxidative stress in DS has not been documented before.This study shows significant correlation of macrocytosis with MDA values. In this study,the increase in MCV also correlated significantly with CAT and uric acid values ,which has not been documented before.

Correlation of macrocytosis with MDA values has been studied in alcoholism18 .In alcoholism, acetaldehyde and MDA form stable aldehyde–hemoglobin condensates .The site of haemoglobin that is modified by aldehyde is located near the erythrocyte membrane . Antibodies to acetaldehyde modified erythrocyte membrane proteins in peripheral blood and bone marrow aspirates brings about the morphological changes such as macrocytosis in chronic alcoholism18 .Similar reason may be producing macrocytosis in Down syndrome .Moreover , such aldehyde bound RBC bring wide spread toxic effects due to oxidative stress18.

Previous studies has been done on macrocytosis in Down syndrome and its association with Vit B12 and Folic acid

levels12,15.David et al in his study on DS concluded that macrocytosis could be due to altered folate remethylation pathway secondary to cystathionine beta synthase overactivity in ch21 as they could not find a significant association of macrocytosis with RBC folate or Vit B12 values12 . Nancy et al also could not find any significant association of macrocytosis with folate concentration in DS15.

The MCV , in this study did not show any significant association with TSH values eventhough it showed correlation with FT3 and FT4 values.Autoimmune hyper and hypothyroidism is common in Down syndrome and macrocytosis does occur in hypothyroidism according to earlier studies19,20. The cause for macrocytosis in DS was postulated to be due to alteration in red cell membrane lipid distribution21,22 and also to vit B12 and folic acid deficiency,secondary to hypothyroididsm23.

Eventhough the Hemoglobin levels was decreased in DS, it was not statistically significant . But the RBC count was significantly decreased in DS and showed significant correlation with MDA, UA , and catalase. The negative correlation with MDA could be due to reduced red cell survival due to increased oxidative stress , causing accelerated aging of RBC as suggested by Wachtel et al11 or due to autoimmune destruction of aldehyde bound cells as suggested by Latvala et al24.

The negative correlation of RBC count with uric acid could be due to increased destruction of RBC producing increase in uric acid level as it is the final product of purine metabolism in humans.RBC count in this study did not show any association with Thyroid status.

The RDW indicates the degree of anisocytosis and it was significantly increased in the DS in this case control study. The RDW showed positive correlation with MDA values25 , Uric acid 26 as in other previous studies.

The significant Leucopenia, Neutropenia in DS in this case control study is in accordance with previous reports12,14-16. The WBC count, lymphocyte count and neutrophil count showed significant correlation with oxidative stress markers ,but failed to show any association with thyroid status.The role of aldehyde protein adducts as a cause for leucopenia in Down syndrome need to be studied in the upcoming research. The decreased count and function of leucocytes makes the DS susceptible for infection due to low immunity27.

Platelet count was significantly decreased in DS and showed significant negative correlation with oxidative stress markers. This has been suggested due to autoimmune destruction of platelets by increased oxidative stress15,16.

CONCLUSION

Macrocytosis ,neutropenia and thrombocytopenia is significantly associated with Down syndrome in this case control study. The haematologic variables showed significant association with oxidative stress markers but failed to show a significant association with thyroid status. Hence as observed from this study and in the light of previous studies on oxidative stress and thyroid status , it should be assumed that the haematological abnormalities in DS is primarily due to significant oxidative stress. Macrocytosis may have a role as an index of oxidative stress in DS as well as in other disorders caused by oxidative stress, where other causes of macrocytosis has been ruled out.

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