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Antioxidant activity in Egyptian children with Down syndrome before and after nutritional supplementation

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ABSTRACT

Down syndrome (DS) is a chromosome abnormality with specific clinical symptoms and mental deficiencies caused by trisomy of chromosome 21. Although the genetic changes cannot be cured, control of the associated symptoms may improve the patients' quality of life. Evidences indicate oxidative stress may play a role in some of the degenerative changes seen in DS. The present study aimed to evaluate red blood cell antioxidant capacity and redox cycle enzymes activities before and after nutritional supplementation, in an attempt to protect DS children from premature degeneration. The study included 21 children with DS, their age ranged from 1 month to 5 years received mixture of nutritional supplements (formula X) for six months and 20healthy matched children served as controls received placebo. Reduced glutathione (GSH), in addition to antioxidant enzymes activities[superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), glutathione reductase (GR), and the glutathione-S-transferase (GST)] were assessed to all participants. Before nutritional supplementation, GSH and GST levels were significantly low, while SOD levels in DS children were significantly high compared to controls. After 6 months' supplementation, significant high levels were observed in the activity of GPx and CAT with elevated GPx/GR ratio and reduced SOD/(CAT + GPx) ratio among DS children. Supplementation of DS children with formula X is important at early years of life, as it may protect against harmful oxidative damage.

Keywords: Down syndrome, Antioxidant enzymes, Egyptian, children, Nutritional supplementation.

INTRODUCTION

Down syndrome (DS) is the most common genetic cause of human mental retardation with a frequency of approximately 1 in 600 to 1,000 live births in Egypt[1].The disease is associated with mental retardation, immune system disorders, and autoimmune processes. It is further characterized by increased incidence of heart defects, gastrointestinal anomalies, and malignancies. Typical manifestations of DS are premature aging and Alzheimer disease as early as ages 30-40 years[2].

There is a primary defense system against oxidative stress, mediated by sequential enzymatic reactions. In the first step of the process, CuZn-superoxide dismutase (SOD1) catalyzes the dismutation of O_2^- to H_2O_2. Glutathione peroxidase (GPx) and catalase (CAT) then independently convert H_2O_2 to water [3]. Any increase in SOD1catalytic activity, therefore, produces an excess of H_2O_2 that must be efficiently neutralized by either GPx or CAT.

Otherwise, H_2O_2 reacts with O_2, producing $OH^{\cdot}$ **,** which is one of the most active reactive oxygen species. Thus the activity of the first-step (SOD1) and second-step (GPx, CAT) antioxidant enzymes must be balanced to prevent cell damage. Genetic over-expression of anti-oxidant enzymes leads to over-consumption of their enzymatic substrates and over-production of their metabolic end-products. In DS individuals, an elevated level of SOD is a leading factor to increased lipid peroxidation and oxidative damage to DNA [4]. Several evidences suggested that oxidative stress may be involved in the premature neuronal degeneration, the cerebral cortex from fetuses with DS was found to have increased SOD activity[5]. Also, cortical neurons from fetuses with DS have an increased concentration of intracellular oxygen-derived free radicals and increased lipid peroxidation [6] and *in vitro* fetal neurons in DS show increased apoptotic degeneration[7].

Glutathione (GSH) plays a fundamental role in numerous metabolic and biochemical reactions such as DNA synthesis and repair, protein synthesis, prostaglandin synthesis, amino acid transport and enzymes activation. Thus, every system in the body can be affected by the state of the glutathione system, especially the immune system, the gastrointestinal system and the nervous system[4].GSH is also considered as an antioxidant as it has an important role in detoxification of free radicals. GSH appears to be decreased in the blood of DS children[3,4].

Glutathione S-transferase (GST) is a family of isoenzymes serving a major role in the biotransformation of many reactive compounds; catalyze the conjugation of GSH with a wide variety of organic peroxides to form more water-soluble compounds for more efficient elimination of toxic radicals [8].

The aim of this study was to evaluate of the effect of mixture of nutritional supplements on the activity of the antioxidant enzymes in DS children which may protect against harmful systemic reactive oxygen species. This may be of great importance in the management of this disorder.

EXPERIMENTAL SECTION

Participants

The present study included 21 patients with DS (males and females) aged 1 month to 5 years. All cases had trisomy 21 karyotype. All patients were selected from the attendants of the Children with Special Needs clinic, National Research Centre, Giza, Egypt. A comparable 20 healthy children were selected and enrolled in the study as control group. DS children were divided into two age categories: Group 1 included 10 cases less than one year and group 2 included 11 cases from 1-5 years.

Ethics approval and consent:

A written consent was obtained from the parents of each participant, according to the guidelines of the ethical committee of the National Research Centre.

Product dosage

The supplement studied was Formula X consisted of three parts, a daily supplement containing (vitamins, minerals, amino acids and related substances) (Table 1), an enzyme supplement (Table 2), and a night-time supplement (Table 3). Nutrients shown were based on 8.2 gm dose (Table 4). Formula X supplement was from International Nutrition, Inc., Baltimore, MD, U.S. DS children received the formula daily according to body weight for six months, while the control children received placebo.

Sample Collection and Analytical Procedures

Venous blood samples were collected in EDTA tubes (1 mg EDTA/1 mL blood) and blood was centrifuged immediately after withdrawn. The erythrocytes were washed three times with a saline solution (9g/l) and hemolyzed by the addition of an equal volume of ice-cold distilled water to yield a 50-percent hemolysate. The erythrocyte lysate was divided into aliquots of 500 µL each and then frozen at -20ºC. Hemoglobin was determined in the whole blood samples using a kit obtained from Sigma Chemicals Co. GSH were measured in hemolysate using the Saville method[9];buffers were prepared according to Gomori [10]. GST activity was assayed with the aromatic substrate 1-chloro-2,4-dinitrobenzene (CDNB) and co-substrate GSH as described by Habdous et al.[11]. GR activity was assayed and expressed in terms of micromoles of NADPH oxidized/g Hb/minute as described by Zanetti[12]. GPx activity was assayed as described by Paglia and Valentine[13], using GSH as a substrate.CAT was assayed at 37°C with H_2O_2 as a substrate according to Aebi[14]. SOD activity was assayed by using the RANSEL kit[15]. All spectrophotometeric measurements were performed in a Shimadzu UV-2401 PC spectrophotometer.

Table 1: Daily Supplement Formula

Ingredients	Units
Vitamins	
Vitamin A (beta-carotene)	3000 IU
Vitamin A (palmitate)	5000 IU
Vitamin D3	75 IU
Vitamin E	400 IU
Biotin	0.2 mg
Folic acid	800 mcg
Niacinamide	125 mg
Pantothenic acid	45 mg
Vitamin B1	55 mg
Vitamin B12	90 mcg
Vitamin B2	45 mg
Vitamin B6	35 mg
Vitamin C	1000 mg
Minerals	
Calcium (citrate)	25 mg
Choline bitartrate	800 mg
Chromium	75 mcg
Iodine	7 mcg
Magnesium	150 mg
Manganese	1.5 mg
Molybdenum	75 mcg
Potassium	15 mg
Selenium	45 mcg
Zinc	10 mg
Acetyl-L-carnitine	45 mg
Amino Acids and related substances	
L-Citrulline	70 mg
L-Glutathione	150 mg
L-Histidine	25 mg
alpha-Ketoglutaric acid	500 mg
L-Methionine	50 mg
L-Ornithine	100 mg
L-Proline	100 mg
L-Serine	150 mg
L-Tryptophan	50 mg
L-Tyrosine	100 mg
Betaine hydrochloride	60 mg
Bioflavonoids	150 mg
Bromelain	5 mg
Coenzyme Q10	30 mg
Inositol	75 mg
Paba	75 mg
Papain	5 mg
Taurine	200 mg
Alpha-Lipoic acid	45 mg

Table 2: Daily Enzyme Supplement Formula

Ingredients	Units
alpha-Amylase	25 mg
Cellusase	1 mg
Lactase	1 mg
Lipase	25 mg

Table 3: Night-time Supplement Formula

Ingredients	Units
Vitamin B6	2.5 mg
L-Ornithine	150 mg
L-Tryptophan	125 mg

Table 4: Percentage of Each Consumed Listed Nutrient

Percentage of each Listed Nutrient	
Up to 20 lbs (≤ 9 kgs)	17 %
21 - 40 lbs (10 - 18 kgs)	33 %
41 - 60 lbs (19 - 27 kgs)	50 %
61 - 80 lbs (28 - 36 kgs)	75 %
Over 80 lbs (>37 kgs)	100 %

(Nutrients shown are based on 8.2 gm dose)

Statistical analysis

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS/Windows Version 9.05, SPSS Inc., Chicago, IL, USA).Student's *t*-test was used; data were expressed as mean ± SE, *p*-values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

The present study showed the following results before taking formula X, significant increase of SOD activity in DS patients ($p<0.01$), significant decrease of GST and GSH activities in DS patients ($p<0.01$) compared to controls. On the other hand no change in GPx, GR and CAT levels were detected. After taking formula X, for six months level of GSH, SOD, GR, and GST showed no statistical difference. However, CAT and GPx level is significantly increased ($p<0.05$, $p<0.01$ respectively) (Table 5) (Figure1,2).

Table 5: Mean Levels of Investigated parameters in Controls and DS Children

Group	SOD (U/gHb)	CAT (KU/g Hb)	GPx (U/g Hb)	GR (U/g Hb)	GST (U/g Hb)	GSH (µmol/g Hb)
Controls	705±65	391± 56.4	19±0.6	2.92±0.235	6.60±0.69	10.3±4.2
Down syndrome (pre)	1472 ±171	427±51.4	23.2 ±2.32	2.99± 0.337	2.28±0.334	7.05 ±0.652
p1	<0.01*	>0.05	>0.05	>0.05	<0.01*	<0.01*
Down syndrome (post)	1510±196	650±87	45.4±5.44	2.79± 0.342	1.96±0.25	7.59±0.532
p2	>0.05	<0.05*	<0. 01*	>0.05	>0.05	>0.05

Each value represents the mean±SE; controls (n=20) ; Down syndrome (n=21)
P1:DS versus control; P2: DS (Pre) versus DS (Post)
*p >0.05=nonsignificant; p<0.05=significant**

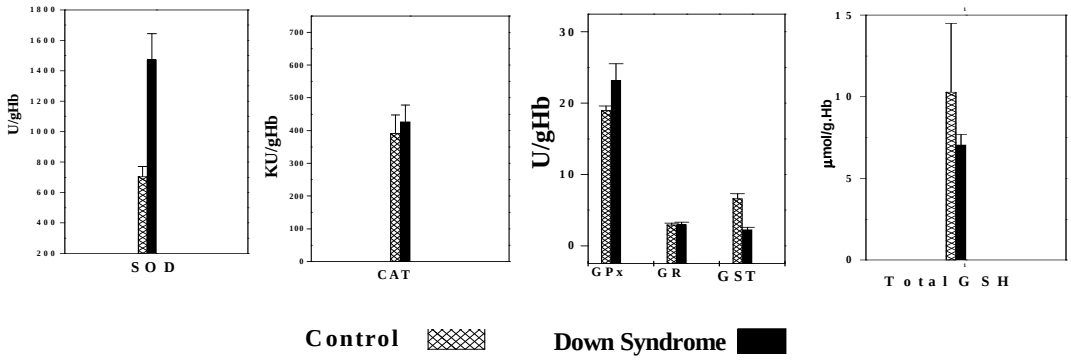

Fig. 1: Antioxidant enzymes activities in DS Children and Controls

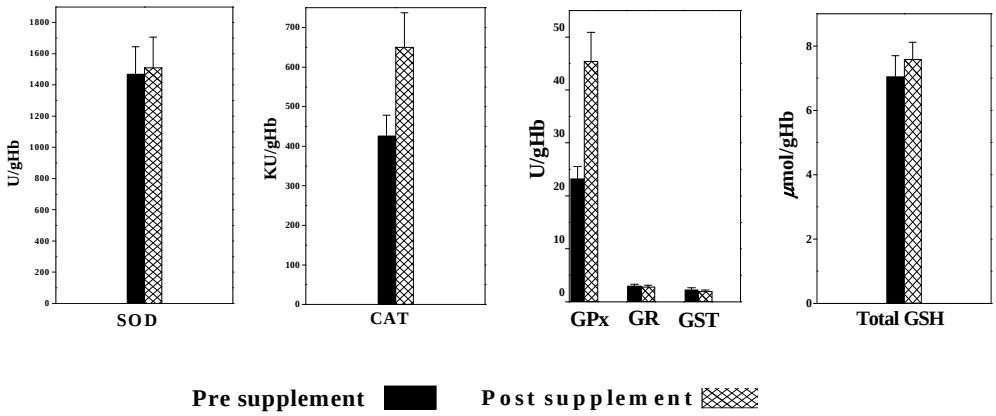

Fig. 2: Antioxidant enzymes activities and in DS Children Pre- and Post-nutritional Supplementation

A significant difference was observed in CAT between group 1 and group 2 in DS patients ($p<0.05$)**.** On the other hand, no statistically significant differences were present neither in the activities of the other enzymes tested nor in the level of RBCs GSH (Table 6).

Table 6:Mean Levels of the Investigated parameters of DS Groups

Down syndrome	Group 1 Less than year	Group 2 1-5 years	T- value	p value
SOD (U/g Hb)	1425±187.8	1644±206.7	0.7842	>0.05
CAT (KU/g Hb)	588±70.62	391±56.4	2.181	<0.05*
GPx (U/g Hb)	22.38±2.11	24.54±3.022	0.5876	>0.05
GR (U/g Hb)	3.534±0.46	2.93±0.235	1.1799	>0.05
GST (U/g Hb)	2.7±0.356	2.18±0.402	0.97014	>0.05
GSH(µmol/g Hb)	7.364±0.7	7.32±0.7	0.04073	>0.05

*Each value represents the mean± SE; p>0.05 = non significant; p<0.05 = significant **

The calculated data of the enzymatic activity in DS groups SOD/GPX 63.4 and SOD/GPX + CAT 3.27 were decreased to 33.3 and 2.17 respectively after supplementation. These ratios almost approach the normal values (37 and 1.27 respectively) however; GPX/GR ratio is increased from 7.78 to 16.29 after supplementation (Table 7).

Table 7: Changes in the Ratio of the Antioxidant Enzymes in DS Children, Pre- and Post-nutritional Supplementation

Down syndrome	SOD/ GPx	SOD/GPx +CAT	GPx/ GR
Presupplement	63.4	3.27	7.78
Post supplement	33. 3	2.17	16.29
%	47.47	33.64	109.4
Controls	37	1.27	6.2

No significant difference was observed between male and females DS patients in all the biochemical parameters.

Supplementations may be one of the most promising treatment methods for DS patients, but no scientifically proven diet or drug is yet available. Nutritional therapies were proposed for DS and usually focusing on one or two items, mixtures that included vitamins, hormones and enzymes were advocated by many authors. MSB Plus(Nutri-Chem Labs) and NuTriVene-D, (International Nutrition, Inc.) are formulas have more than 40 ingredients, most of them the same or similar, but with differences in dosage. Various formulas from other companies have not been as popular and reviewed by Roizen[16]. Measures of oxidative damage in DS patients during the early years of life demonstrated increased lipid and DNA oxidation biomarkers as compared with their non-DS siblings [17].

In the present study, the concentration of GSH was significantly low in DS groups, compared to controls. The deficiency of GSH observed in DS patients may be attributed to increased oxidation to GSSG, increased degradation or decreased synthesis of GSH. Pogribna et al. [18]reported that plasma GSH levels were significantly low in the DS children that may reflect an increase of oxidative stress due to over expression of the superoxide dismutase gene

located on chromosome 21.Also over expression of Cystathionine beta synthase in DS, located on chromosome 21 indirectly lead to reduction of methionine and regeneration of cysteine which is the rate limiting amino acid for glutathione synthesis [19]. However, supplementation of DS patients with Formula X containing L-glutathione (150 mg) and the amino acids methionine (50 mg) and α- ketoglutaric acid (500 mg) required for GSH synthesis did not elevated the reduced GSH level in RBCs.

Our study showed high level of SOD activity in DS patients compared to controls. Similar results was observed by Pastore et al. [3]who reported elevated SOD level 50-percent higher than normal in a variety of DS cells and tissues, including erythrocytes, B- and T-lymphocytes, and fibroblasts. No significant difference in SOD activity level was observed between the two groups of DS patients. These data are consistent with the study conducted by Muchova et al.[20] who found that SOD activity in DS patients did not change significantly with age. In the present work, supplementation with Formula X for 6 months did not significantly affect the SOD level in DS children.

CAT activity level was high in DS patients compared to controls but with no significant difference, significant increase in CAT activity levels in DS patients was noticed after supplementation with Formula X.

Significant high levels of CAT activity were observed in group1 DS compared to group 2; this is consistent with Casado and López-Fernández [21]who reported high CAT activity levels in newborns and low activity levels in children between 1-9 years. McElroy et al. [22]reported that CAT is the only antioxidant increased in activity with progressing gestational age.

In our study, no significant difference was observed in GPx activity level in DS children compared to controls or between both groups of DS patients. These results are in agreement with the study conducted by Muchova [20]and Shawky[23]who reported that the oxidative imbalance in trisomic cells is age-dependent and depends on the ratio of SOD to (CAT + GPx) rather than on absolute amounts of these antioxidant enzymes. An imbalance in these enzymes may have adverse effects on cell membranes via the indiscriminate oxidation of susceptible molecules such as polyunsaturated fatty acids (PUFAs). De Haan et al. [24]and Percy et al. [25],reported that this alteration in this ratio is representing an important determinant of cellular damage, because changes in this quotient correlate well with an increase in lipid damage. The erythrocytes of DS children, adolescents, and adults exhibited systemic increases in SOD, SOD/GPx or the SOD/(GPx +CAT) activity ratio[26]. This disequilibrium may contribute to some of the pathologic features occurring in the DS phenotype [3]. Meguidet al. [27]reported an increase in whole-blood GPx level in DS patients (8 month-3years) with complete trisomy, which caused by abnormal cell division during development of the ovum or sperm or during fertilization and represented by 95% of all cases, and translocations (partial trisomy 21), which caused by break off the extra chromosome 21 and become attached to another chromosome representing by 3%- 4%. The balance in the SOD/GPx ratio is an important determinant of cellular aging and increased with the severity of the stress.

The ratio of SOD/(GPx+CAT) in our study was decreased after supplementation with formula X for six months in DS children from 3.27 to 2.17,. In contrast to our results, Ellis et al.[7] reported non-significant effect of only antioxidant supplementation on SOD or GPx activities or on the SOD/GPx ratio in DS infants under age seven months.

In a study by Antila et al. [28] applied 15-25 mcg selenium/kg/day to seven DS patients (ages 1-54 years) resulted in a 25-percent increase in GPx activity and a 24-percent reduction in the SOD/GPx ratio. Anneren et al. [29] reported that oral selenium supplementation to DS patients reduced the rate of infections and had immunoregulatory effects in DS subjects.

Our results showed non-significant difference in GR activity between DS patients and controls before and after nutritional supplementation. Concentration of GSH is partly regulated by GPx and GR activity. The decreased activity of GR together with the increased GPx activity could lead to a reduced GSH/GSSG ratio during the aging process and could explain age-associated decrease in blood GSH levels [8].In a study conducted by Erden-Inal et al. [30], they reported that glutathione redox system is affected in the first years of life and aging periods in healthy individuals. Also, Pastor et al found that GR catalytic activity decreased with age in DS groups, with significant differences between the young and old age groups[31]. However, neither Muchova [20]nor Pastore [3] found significant differences in GR catalytic activity in DS compared to controls, our results are in agreements with these latter findings.

The GSH/GSSG ratio is considered a measure of oxidative stress. In erythrocytes, the GPx/GR catalyzing GSH/GSSG cycle plays a key role in metabolizing H_2O_2 and organic peroxides. Imbalance of this ratio creates oxidative stress. Pallardó et al.[32]reported that the GSSG/GSH ratio was significantly high in young DS patients in contrast to DS patients aged ≥15 years who had significantly lowers GSSG/GSH ratio compared to controls of the respective age groups. In our study supplementation with formula X elevated the GPx/GR ratio from 7.78 to 16.29,this increase in this ratio is directly related to the increase of GPx and not GR.

Neonates are exposed to oxidative stress and cell damage as a result of a sudden increase in oxygenation at birth; therefore, GST activity in adults is lower than GST activity in neonates [33]. Zhong et al.[34]reported that there was no difference in GST activity between ages 21-81 years, and gender in healthy Chinese subjects, although there was a trend for men to have higher GST activity than women. This is inconsistent with the data reported by other researchers where no gender differences in GST activity were observed [35]. Our data revealed that the activity of GST in the DS group was significantly lower than controls. This result is consistent with Pastore et al. [3] who reported decrease in catalytic activities of GST in DS children compared to controls. However Cengiz et al. [36] studied erythrocytes of DS patients (over age 14 years) and reported no significant changes in GST and GSH levels between the DS and controls.

No gender difference in SOD, GPx, CAT, GR, GST, or total GSH were detected in DS children, in our study supporting the results of Muchova et al.[20] Casado and López-Fernández [21] and Shawky et al.[23].On the other hand, Anneren et al reported higher GPx level in girls than in boys with DS [29].

CONCLUSION

Nutritional supplementation with Formula X at early ages may play an important role in protecting against oxidative damage in Ds patients. Further studies are needed with large sample size and for long duration, as it may be too optimistic to expect subtle physiological improvements to be translated into detectable physical and mental changes.

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REFERENCES

[1]NAMeguid;AADardir;M Khass;LE Hossieny ; A Ezzat ; MK El Awady,*Disease Markers*,**2007**, 23, 1–8.
[2]AGhezzo ; S Salvioli ; MC Solimando ;A Palmieri ; C Chiostergi ; M Scurti ; Lomartire L; Bedetti F; Cocchi G; Follo D; Pipitone E; Rovatti P; Zamberletti J; Gomiero T; G Castellani ; C Franceschi,*PLoS One*, **2014,** 9(11), e113111.
[3] A Pastore;G Tozzi;LM Gaeta;A Giannotti ; E Bertini ; G Federici ; MC Digilio ; F Piemonte,*J. Pediatr*.,**2003**, 142,583-585.
[4] MJ Shichiri, Clin Biochem Nutr., **2014,** (3),151-60.
[5]BW Brooksbank;R Balazs,*Brain Res*.,**1984**,318,37-44.
[6]J Busciglio;BA Yankner,*Nature*,**1995**, 378,776-779.
[7] JM Ellis;HK Tan;RE Gilbert; DP Muller ; W Henley ; R Moy ; R Pumphrey ; C Ani ; S Davies ; V Edwards ; H Green ;A Salt ; S Logan,*BMJ*.**2008**, 336,594-597.
[8]I Ceballos-Picot;JM Trivier;A Nicole; PM Sinet ; M Thevenin,*Clin. Chem*.,**1992,** 38 (1),66-70.
[9]B Saville,*Analyst*,**1958**, 83, 670-672.
[10]G Gomori,*Methods Enzymol*.,**1955**; 1,138-146.
[11]M Habdous;M Vincent-Viry;S Visvikis;G Siest, *Clin. Chim. Acta*.,**2002**,326, 131–142.
[12]G Zanetti,*Arch. Biochem. and Biophsics*.,**1979**, 198(1),241-246.
[13]DE Paglia ;WN Valentine,*J. Lab. Clin. Med*.,**1967**, 70 (1),158-169.
[14]HE Aebi , Catalase. In: Bergmeyer HU, ed. *Methods of enzymatic analysis*. Vol. 3 Deerfield Beach, FL: Verlag Chemie; **1983**, 273–386.
[15]JM McCord;I Fridovich,*J. Biol. Chem*.,**1969**, 244,6049-6055.
[16]NJ Roizen,*Mental Retardation and Developmental Disabilities*,**2005**, 11,149-155.
[17]EB Parisotto ; TR Garlet ; VL Cavalli ; A Zamoner ; JS da Rosa ; J Bastos ; GA Micke ; TS Fröde ; RC Pedrosa ; D Wilhelm Filho,*Res Dev Disabil*., **2014,** (6),1228-36
[18]M Pogribna;S Melnyk;I Pogribny; A Chango ; P Yi ; SJ James,*Am. J. Hum.Genet*., **2001**, 69, 88–95.

[19]SJ James;P Cutler;S Melnyk;RJ Hine ; JB Gibson ; P Yi ; DL Tafoya ; DH Swenson ; VL Wilson; DW Gaylor ,*Am. J. Clin. Nutr*.,**2004**,80,1611–1617.
[20]J Muchová;M Šustrová;I Garaiová;A Liptáková ; P Blazícek ; P Kvasnicka ; S Pueschel ; Z Duracková,*Free Rad. Biol. Medi*.,**2001**, 31(4), 499-508.
[21] A Casado ;M E López-Fernández,*Gerontology* **2003**, 49(4), 251-254.
[22]MC McElroy ;AD Postle ;FJ Kelly,*Biochim. Biophys. Acta*,**1992**,1117,(2),153-158.
[23]RM Shawky;EA Abdelaziz;RM Elhossiny;ME Ahmed,*Egypt. J. Med. Human Gene*., **2004**,5(1),103-109.
[24]JB De Haan;F Cristiano;RC Lannello;I Cola,*Biochem. Mol. Biol. Int.*,**1995**,35, 1281–1297.
[25]ME Percy;AJ Dalton;VD Markovic;DR McLachlan ; JT Hummel ; AC Rusk ; DF Andrews, *Am. J. Med.Genetics.*,**1999**, 35, 459–67.
[26]G Capone,*Developmental and Behavioral Pediatr*.,**2001**, 22(1), 40-59.
[27]N Meguid ;NM Kholoussi;HH Afifi. *Biol. Trace Elem. Res.*,**2001**, 81,21-28.
[28]E Antila;URNordberg; ELSyvaoja; T Westermarck,*Adv. Exp. Med. Biol*., **1990**,264,183–186.
[29]G Anneren;CG Magnusson;SL Nordvall,*Arch. of Disease in Childhood*,**1990**, 65,1353–1355.
[30]M Erden-Inal ;E Sunal ;G Kanbak ,*Cell Biochem. Funct.*,**2002**,20(1),61-66.
[31]MC Pastor;C Sierra;M Dolade;E Navarro;N Brandi ; E Cabré ; A Mira ; A Serés , *Clin. Chem*.,**1998**, 44, 924–929.
[32]FV Pallardó;P Degan;M d'Ischia;FJ Kelly ;A Zatterale ; R Calzone ; G Castello ; R Fernandez Delgado ; C Dunster ; A Lloret ; P Manini ; MA Pisanti ; E Vuttariello ; G Pagano,*Biogerontology*,**2006**, 7 (4), 211-220.
[33]B Porto;RJD Oliveira;C Sousa;J Gaspar ; J Rueff ; F Carvalho ; I Malheiro,*Mutation Res.*,**2006**, 603, 41–47.
[34]SLZhong;SFZhou;X Chen; SY Chan;E Chan ; KY Ng ;W Duan, M Huang *Eur. J. Pharmaceutical Sci*.,**2006**, 28,77-85.
[35]S Habifa;I Mutaf ;N Turgan;E Onur ; C Duman ; D Ozmen; O Bayindir,*Clin. Biochem.*,**2001**, 34, 667–671.
[36]M Cengiz;M Seven;N Suyugul,*Genet.Couns.*,**2002**, 13(3),339-342.