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Micronucleus assay and pro-oxidant status of patients with known chromosomal aneuploidy

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ABSTRACT

Aneuploidy is a well known chromosomal abnormality, and it is considered as a major cause of congenital birth defects and miscarriage. The present study aims to examine micronucleus frequency (MN), oxidative stress and antioxidant activity in chromosomal aneuploidy disorders including Down syndrome (DS), Klinefelter (KF) and Turner syndrome (TS), comparing to healthy controls matched age group. Peripheral blood samples were collected from 50 subjects, including, 20 normal healthy control and 30 patients with Down, Klinefelter and Turner syndromes. Micronucleus level was scored for each subject. Biochemical parameters, including lipid peroxide (LPO), nitric oxide (NO), total antioxidant capacity (TAC) were determined .The activity of paraoxonase1 (PON1) was also detected in blood serum for the three genetic diseases. DS and KF syndrome cases had an elevated count of MN (13.27 ± 0.59, 14.30±1.29 respectively vs. 1. 80 ± 0.19; P < 0.05). However, Turner patients recorded an insignificant change in micronucleus frequency. In addition, significant decrease in TAC and PON1 was recorded in all patients comparing with normal controls (p≤0.05). While, NO and MDA product declared significant increase in DS, KF and Turner syndrome as compared to normal control group. Thus, it could be concluded that the MN, antioxidants and oxidative stress biomarkers assays are a powerful tools for detecting genetic disorders in chromosomal aneuploidy patients.

Keywords: Down syndrome, Klinefelter syndrome, Turner syndrome, Micronucleus frequency and lipid peroxidation,

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INTRODUCTION

Aneuploidy is considered as the most common chromosomal abnormality, and it is the major cause of congenital birth defects and miscarriage [1, 2]. Down syndrome (DS) is a genetic syndrome of chromosomal origin [3]. In addition to mental and congenital disorders, DS individuals exhibited increased risk of malignant disease and have premature aging symptoms, including high level of oxidative stress [4], instability of genome[5], apoptosis[6], can-

cer[7], and Alzheimer's disease (AD) [8]. Thyroid impairment is the most representative endocrine anomaly in patients with DS. It is well understand that thyroid impairment is extremely predominant in children and adults with DS and that both hypothyroidism and hyperthyroidism are highly spread in patients with DS than in the generic population. Growing index has shown that DS patients are extending remarkable elevated oxidative stress, which may be engaged in the great propagation and seriousness of a many of pathologies connected with the syndrome, as well as the quickened senility noticed in these patients [9]. However, Sulthana et al.[10] declared that, no significant difference in the antioxidant enzyme activities was detected, whereas the ratio of SOD -1 to CAT and GPx (SOD-1/CAT+GPx) was significantly elevated indicating oxidative stress in Down syndrome.

Whereas Klinefelter (KF) is a disease associated with less production of testosterone, failure in testicular function and spermatogenesis. This syndrome is due to sex-chromosome disorder as KF syndrome has an additional X chromosome to become (47, XXY)[11]. Oxidative destruction is a leading cause for the 30-80 % of all cases of male infertility. The reactive oxygen species (ROS) can be produced from environmental factor, infectious and life style condition. Oxidative stress can be determined during the clinical evaluation of the infertile male using immediate and unmediated measurement. Indirect method determined the net results of imbalance between ROS initiation and the antioxidants that trap ROS, by measure sperm cell membrane oxidation[12]. The most common method is measuring malondialdehyde (MDA), which is the final products of lipid peroxidation of sperm cell membrane. Determination of DNA destruction in male infertility has also been implicated as an indirect method of intracellular ROS stimulated oxidative impairment[13].

Turner syndrome (TS) is a popular sex chromosome aberration in women, characteristic by loss of the X chromosome or an important part of it [14]. The conventional karyotype 45, X is responsible for more than 50% of cases[15]. However, Turner syndrome female showed that hypo and hyper-thyroidism, diabetes type 1, celiac, and inflammatory bowel disorders resulted from oxidative –mediated damage were markedly spread[16]. We therefore, hypothesized that all these disorders are associated with genetic and oxidative destruction. Thus, this study aims to evaluate the frequency of micronucleus in different patients of chromosomal aneuploidy such as DS, KF and TS, as a high benefit controlled method for detection of genetic damage, as compared to healthy control group.

The micronucleus assay is considered as one of the most common methods in genotoxicity testing. Micronuclei are complete chromosomes or fragments, which excluded from the nucleus. Many theories may be included in the formation of micronuclei as chromosome breakage (clastogenesis) and spindle disruption (aneuploidogenesis) [17].

MATERIALS AND METHODS

Peripheral blood samples were collected from 50 subjects, 30 patients with Down, Klinefelter and Turner syndromes and 20 normal healthy controls. Once the principal clinical aspects of the different syndromes were defined, questions were formulated with the intent to then develop recommendations that addressed these questions. All patients were all non-smokers with no previous history or family history of cancer and not receiving anti-folate therapy. Volunteers did not receive any remuneration for their participation. Micronucleus level was scored for each subject.

The Ethical approval for samples collection was obtained from the Ethical Committee of the National Research Centre, Egypt before starting the experiments.

Cell sampling, preparation and scoring:

Micronucleus assay was carried out as described by (Verma and Babu) [18]. Slides were prepared as one thousand cells per individual were scored for the presence of micronuclei and dicentric bridges (nucleoplasmic bridges between daughter nuclei) according to Fenech M.[19]. While, mono and bi-nucleated cells were scored as per criteria according to Ferreira et al.[8]. The abnormal nuclear morphologies are thought to be indicative of DNA damage and/or various stages of morphogenetic or toxicity-induced cell death. Photographic images showing distinct cell populations scored (Photos 1and 2).

Biochemical parameters:

- **Lipid peroxidation:** Lipid peroxide was determined as malondialdehyde (MDA) and its concentration was calculated using the extinction coefficient value 1.56<105 M-1 cm-1 and read colorimetrically at 535 nm [20].
- **Nitric Oxide (NO)**: was determined in blood serum according to the method of Moshage *et al*,[21].

- **Total antioxidant capacity (TAC):** was determined according to the method described by Koracevic *et al*, [22].
- **Paraoxonase1 (PON1):** activity of was determined by measuring the rate of hydrolysis of substrate paraxone into nitrophenol through the change in absorbance at the wavelength of 412 nm, according the method of Primo *et al*, [23].

Statistical analysis

Analysis of data were carried out using SPSS (one-way analyses of variance), coupled with Co-state computer program (version 8), where unshared latter is significant at $p \leq 0.05$. All data are represented as mean ± SD of 10 cases in each group.

RESULTS

Micronucleus assay

Micronucleus level of DS and KF individuals were significantly higher than control subjects about 13-14 fold ($P \leq 0.05$) (Table 1, Fig.1 and Photos 1 and 2). While, insignificant difference was observed in the micronucleus level between control and TS individuals (Table 1 and Figs 1 and 2).

Table 1: The mononuclear activity (No of MN/1000 cell)

Cases	Mean	Range
Control	1.8 ± 0.19^{a}/1000 cell	0-8/1000 cell
DS	13.27 ± 0.59^{b} /1000	
KF	14.30 ± 1.29^{b} /1000	
TS	$0/1000^{a}$ cell	

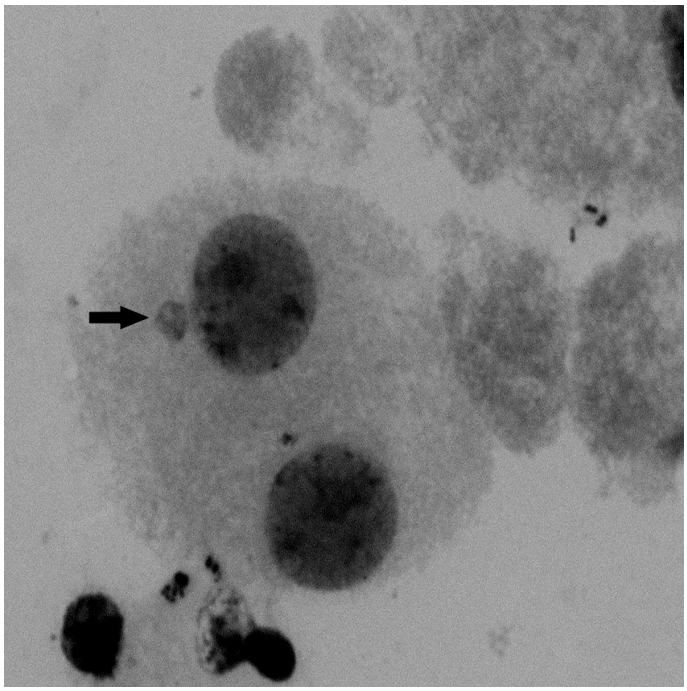

Photo 1: shows bi-nucleated cells with micronucleus as indicated by the arrow in DS patient

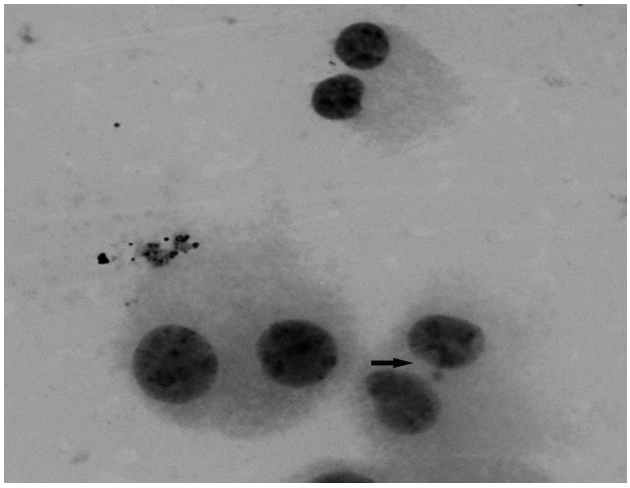

Photo 2: shows bi-nucleated cells with micronucleus as Indicated by the arrow in KF patient

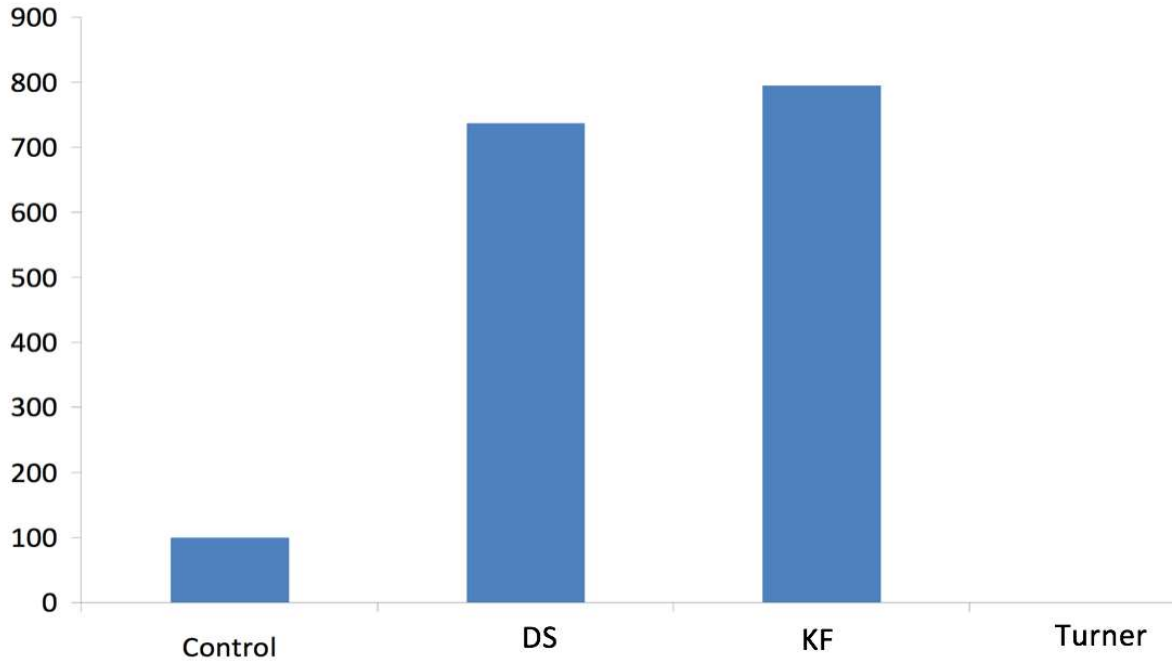

Fig 1: Percentage changes in mononuclear activity in different genetic diseases

Biochemical parameters

The biochemical results clearly demonstrated statistically elevated levels in both MDA and NO levels in DS, KF and TS subjects comparing with normal control subjects. KF disease showed significantly higher MDA level (32%) than DS and TS (26.67 and 23%, respectively) as compared to healthy control subjects. Moreover, there was significant increase in NO level in KF, TS and DS (9.73, 8.93 and 6.67%, respectively) comparing with normal individuals. There were no significant differences in NO levels among KF, DS and TS patients. On the other hand, TAC and PON exhibited significant decreased levels in DS, KF and TS with percentages 40.58, 20.29 and 27.68% respectively. Meanwhile, the reduction percentages of PON1 reached to 11.7, 19.32 and 14.69 %, respectively comparing with normal control group (Table 2 and Fig.1, 2).

Table 2: Oxidative stress biomarkers and total antioxidant capacity levels in different chromosomal aneuploidy diseases

	Control	Klinefelter Syndrome	Turner Syndrome	Down Syndrome
TAC (umol/L)	0.69 ± 0.03[a]	0.41 ± 0.02[b]	0.55 ± 0.13[c]	0.499 ± 0.01[c]
PON (U/L)	201.80±13.62[e]	178.00±8.22[f]	162.80±10.31[g]	172.10±13.80[f]
NO (Umol/L)	75.00±1.83[a]	82.30±2.36[b]	81.70±2.79[b]	80.00±2.748[b]
MDA (Umol/L)	3.00±0.11[a]	3.96±0.10[b]	3.80±0.14[b]	3.69±0.12[b]

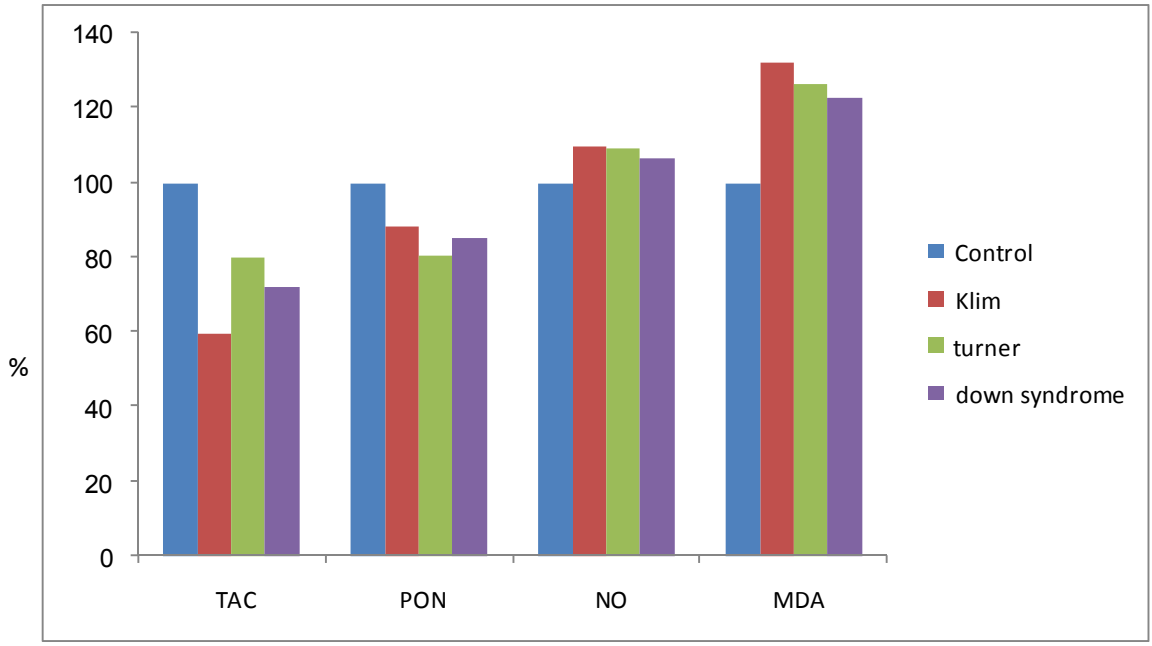

Fig 2: Percentage changes in oxidative stress biomarkers and TAC levels in different genetic disease

DISCUSSION

DS individuals in the present study, showed significant higher Micronucleus level than controls. DS is precisely connected with lower repairing efficiency of DNA. Down syndrome is characterized by impaired cell proliferation, leading to increase frequency of Micronucleus. It has been suggested that cells that fail to complete cytokinesis show a higher frequency of Micronucleus frequency (MN) than healthy counterparts[24] indicating that the frequency of Micronucleus cells could be used as a marker of aneuploidy risk. In agreement with the present results, Thomas et al.[5] declared that the frequency of Micronucleus in the buccal cells of DS patients was higher than that of healthy controls. However, Maluf and Erdtmann [26] showed an insignificant difference among DS subjects and controls. Considering, the association between the increase in (MN) and early Alzheimer symptoms, one could postulate that the elevated MN in different genetic disorders could be attributed to early progression of AD in the DS subjects and may be used as a potential biomarker in characterizing subjects who are at high risk to develop AD[5]. Micronuclei have been shown to be increased with aging in peripheral lymphocytes[8]. In the present study, Micronuclei were statistically increased in the Down's cohort and KF and were estimated to be 13-14 fold higher than in healthy control individuals, confirming the elevated genome damage in these aneuploidy syndromes[25].

It is postulated that the high coding Cu/Zn superoxide dismutase from chromosome 21 in DS patients leads to a disturbance in enzymes balance that are responsible for oxygen radicals' metabolism [26] and thus enhancing the levels of oxidative stress. Free radicals causes damage for DNA and consume the repairing efficiency of cells, causing breakage of chromosome and accumulation of micronucleus, disturbing cell cycle. Similarly, to other genetic disorders, DS and KF are associated with impairment in the DNA repairing mechanisms [27].

DS patients are also susceptible to age related neural degenerative diseases. Excessive Oxidative stress (OS) has deleterious effects on cell membrane structures, nucleic acids, lipid metabolism and protein function and can lead to premature cell aging and cellular dysfunction[27]. The significantly elevated MDA level that was detected in the serum of DS subjects in our study, is consistent with previous reports of Capone et al.[28], who suggested that free

radical-induced lipid peroxidation occurs despite the up-regulation of antioxidants (such as superoxide dismutase) and protective scavengers (as glutathione peroxidase) in DS subjects. High levels of MDA were also observed in the urine and erythrocytes of DS subjects [29]. MDA is believed to be generated under stress conditions and is a potent mutagen and toxic agent in the cardiovascular system. By reacting with DNA, MDA produces DNA adducts that can lead to chromosome mutations if they were not repaired [30]. Elevated levels of MDA in DS patients could result in additive impairments in tissue cells, with a reduced capacity to defend against cellular OS that can lead to a vicious circle of oxidant-induced damage[30].In our study, Patients with DS, KF and TS showed higher serum MDA level than the normal control that suggested its participation in oxidative stress and the disease complication.

Nitric acid (NO) has been related to free radical and reactive oxygen species. In our study, NO levels were statistically increased in all patients comparing to normal controls. NO is an important intercellular signaling molecule that plays a role in the vasodilator responses. High amounts of NO may be synthesized in inflammation, re-endothelization and angiogenesis. NO may mediate vascular damage in the all patients under investigation[20]. The anatomical association of dopamine neurons and nitric oxide synthase-containing interneuron has been previously described[31]. It has been suggested that dopaminergic transmission is modulated by nitric oxide. It was declared that an alteration of the dopaminergic neurotransmission was involved in the pathogenesis of DS and could also be altered as a part of the disturbances in nitric oxide levels[32]. Total Antioxidant Capacity (TAC), is an important biomarker to measure the antioxidant potential of body fluid. The observed reduction in TAC levels that we detected in DS, KF, and TS comparing to control is an indication of a marked OS, this is consistent with the study by Subramaniam et al. [33], where children with DS have a significant reduction in saliva TAC levels along with a significant elevation in NO, an indication of a marked OS.

Paraoxonase1 (PON1) is an HDL-linked enzyme and attributed to HDL protection against coronary artery disease. Significant association between autism and PON1 activity was detected in Caucasian-American[34]. An Inverse relationship was investigated between PON1 mRNA and hyper-homocysteinemia, due to hyper-homocysteinemic diet or a genetic deficiency in cystathionine beta synthase (CBS)[35]. Decreased PON1 could explain the increased lipid peroxidation/oxidative stress observed in autistic patients and could be one of the steps leading to Hcy toxicity in autism[36].

The reduced serum PON1 activity may be due to disturbance in the oxidant –antioxidant imbalance of the proteins free sulfhydryl group, which prevent the inhibition of PON1 activity under oxidative stress [37]. Moreover, PON1 can preserve HDL particle and integrity from oxidation in animal[38]. The low PON1 expression and activity has been also found to be connected with oxidative stress and inflammation conditions linked with coronary heart diseases [39].

Thus, the present results suggested a correlation between PON1 activity, HDL-C, TAC and lipid peroxidation (MDA level) in DS, TS and KF syndrome. These findings pointed out to the priority of PON1 activity and TAC determination in order to examine their participation in these genetic disorders associated with elevated risk of coronary heart disease.

CONCLUSION

It could be concluded that, the micronucleus assay, oxidative stress biomarkers as well as anti-inflammatory activity of paraoxonase1 are considered as a promising testing tools for detecting genetic syndromes in patients with chromosomal aneuploidy like Down, Klinefelter and Turner syndromes.

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