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# CBMN score: A biomarker for genotoxicity in cases with trisomy 21.

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## Abstract

**Down syndrome (DS) is associated with oxidative stress which occurs due to over expression of Superoxide dismutase-1 (SOD-1) gene, located in chromosome 21q 22.1. This results in increased free radical generation causing damage to cellular components. The aim of the study is to quantify the Cytokinesis block Micronucleus score (CBMN) and Total antioxidant status (TAS) in DS and establish a relationship between both. Our results suggest that CBMN frequency (3Vs1) and TAS levels (357.97 Vs 516.3 uM/L) were significantly increased and decreased respectively compared to matched controls. A significant negative correlation was observed between the CBMN score and TAS level. Hence, we conclude that CBMN score can be considered as a biomarker of genotoxicity in cases with trisomy 21.**

**Keywords:** Down syndrome, Oxidative stress, Superoxide dismutase, Cytokinesis block micronucleus assay, Micronucleus, Total antioxidant status, CBMN score, Biomarker, Genotoxicity, DNA damage, Free radical, Reactive oxygen species.

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## Introduction

Down syndrome (OMIM-190685) or Trisomy 21 is a complex metabolic and genetic disorder caused by the presence of three copies of chromosome 21 [1]. There are enough evidences showing unusual oxidative stress among individuals with Down syndrome (DS) which occurs due to over expression of Superoxide dismutase-1 (SOD-1) gene, housed in chromosome 21q 22.1.The relative over production of Superoxide dismutase enzyme encoded by SOD-1 gene leads to disturbance in the equilibrium existing between itself and other antioxidant enzymes (Catalase and Glutathione peroxidase). This imbalance aids in generation of potentially harmful reactive oxygen species (ROS) that causes genotoxic insult to the genetic material in the cell [2].

Micronuclei (MN) are acentric chromosome fragments or whole chromosomes that lag behind at anaphase during nuclear division and are excluded from the nucleus.It is morphologically similar to main nuclei but smaller in size hence the name. Several mechanisms involved in the formation of MN includes chromosome breakage due to oxidative stress induced DNA damage and DNA misrepair, chromosome loss and nondisjunction due to mitotic malfunction [3].The cytokinesis block micronucleus (CBMN) assay is a simple technique and is preferred for demonstrating MN in cultured human lymphocytes.

The antioxidant system includes enzymatic antioxidants, macromolecules such as albumin, ceruloplasmin, and ferritin; micro molecules including Vitamin C, Vitamin E, β-carotene, reduced glutathione, uric acid, and bilirubin. The total antioxidant status (TAS) represents the sum of endogenous antioxidants. The said antioxidants together provide greater protection against attack by reactive oxygen species (ROS), than any single compound alone. Thus, the TAS gives more relevant biological information compared to that obtained by the measurement of individual components, as it considers the cumulative effect of all antioxidants present in plasma. TAS levels are inversely proportional to the oxidant load and pro-oxidant status of an individual and have been found significantly decreased in DS [4,5].

A simple yet sensitive indicator for highlighting the harmful effects of oxidants is essential in subjects with DS. So, the goal of the current study is to evaluate the damage caused by ROS on nuclear material by employing CBMN assay, study the effect of free radicals on antioxidant capacity by measuring TAS and to check the sensitivity of CBMN assay in indicating oxidative stress induced nu-

clear damage by correlating the CBMN score with TAS in DS individuals.

## Material and Methods

The study was conducted in the Cytogenetic unit, Department of Anatomy in collaboration with Department of Pediatrics, JIPMER hospital, Puducherry, India. Study group consisted of hundred and eight (108) clinically diagnosed children with Down syndrome with mean ± SD age of 3.03 ± 4.1 which included 64 males and 44 females. The control group was formed by hundred and ten (110) age and sex matched healthy normal children. Ethical clearance was obtained from Institute Human Ethics Committee, JIPMER hospital, Puducherry. Written informed consent was obtained from the parents/guardians of both case and control group prior to the study.

Peripheral venous blood was collected into a sterile anticoagulant rinsed syringe under aseptic precautions by venipuncture. The diagnosis of Down syndrome was made by clinical features and followed by laboratory confirmation of trisomy 21 by karyotyping and interphase-FISH (TelVysion 21Q, Spectrum Orange, Abott-Molecular, USA) using Olympus BX-51 epifluorescence microscope-Japan and automated karyotyping workstation, Ikaros/Isis Metasystem, Carl Zeiss-Germany [6] [Fig 1].

Phytohaemagglutinin (SIGMA-ALDRICH) stimulated conventional lymphocyte cell culture utilising RPMI-1640 (SIGMA-ALDRICH) was used for carrying out CBMN assay. Whole blood, about 4-5 drops was inoculated into the culture media and incubated at 37ºC for 44 hours. Cytochalasin-B (SIGMA-ALDRICH), inhibitor of actin filament network formation which arrests the cell division at cytokinesis was added to 44 hour cultures at a final concentration of 3µl /ml and reincubated. Cultures were harvested 28 hours after Cytochalasin-B additions following a hypotonic treatment with KCl (0.075 M).

Cells were fixed with 3:1 methanol acetic acid and the slides were stained with Giemsa for three minutes. The CBMN score or micronucleus index for each sample was analysed by counting 1000 binucleated cells based on the scoring criteria outlined by HUMN project [7]. The binucleated cells were scored blindly at 400X magnification under Olympus BX-51

Epifluorescence microscope-Japan. Plasma total antioxidant status was measured by the method proposed by Benzie et al [8].Appropriate parametric (independent students t test) or non-parametric tests (Mann Whitney U) were used for comparing the continuous data between the two groups. Pearson correlation coefficient was used for association between the groups.

All statistical analysis was carried out at 5% level of significance and p value < 0.05 was considered significant. Analysis was done using SPSS (Version 19) software.

## Results

Chromosomal analysis by karyotyping and Interphase FISH (Fig.1) confirmed the presence of pure trisomy 21 in all hundred and eight (108) clinically diagnosed children with DS. Other variants like partial trisomy, translocation and mosaicism were not observed in the present series. Ferric reducing capacity of plasma (FRAP), which was used as an indicator to measure total antioxidant status, showed a significant ($p<0.001$) decrease among cases with DS compared to healthy controls (Table 1). CBMN score was found to be significantly increased ($p<0.001$) in DS compared to controls (Table 1) (Fig-2). When comparing the CBMN score with TAS, a significant negative correlation ($p<0.01$) was noticed between CBMN score and TAS ($r^2 = 0.481$) (Fig. 3).

***Table 1:*** *CBMN score and TAS levels in DS cases and controls*

| | **CBMN score** (median) | **TAS (µM/L)** (mean ± SD**)** |
|---|---|---|
| DS Cases (n=108) | 03 | 357.97 ± 112.13 |
| Healthy Controls (n=110) | 01 | 516.3 ± 112.17 |
| **p value** | < 0.001 | < 0.001 |

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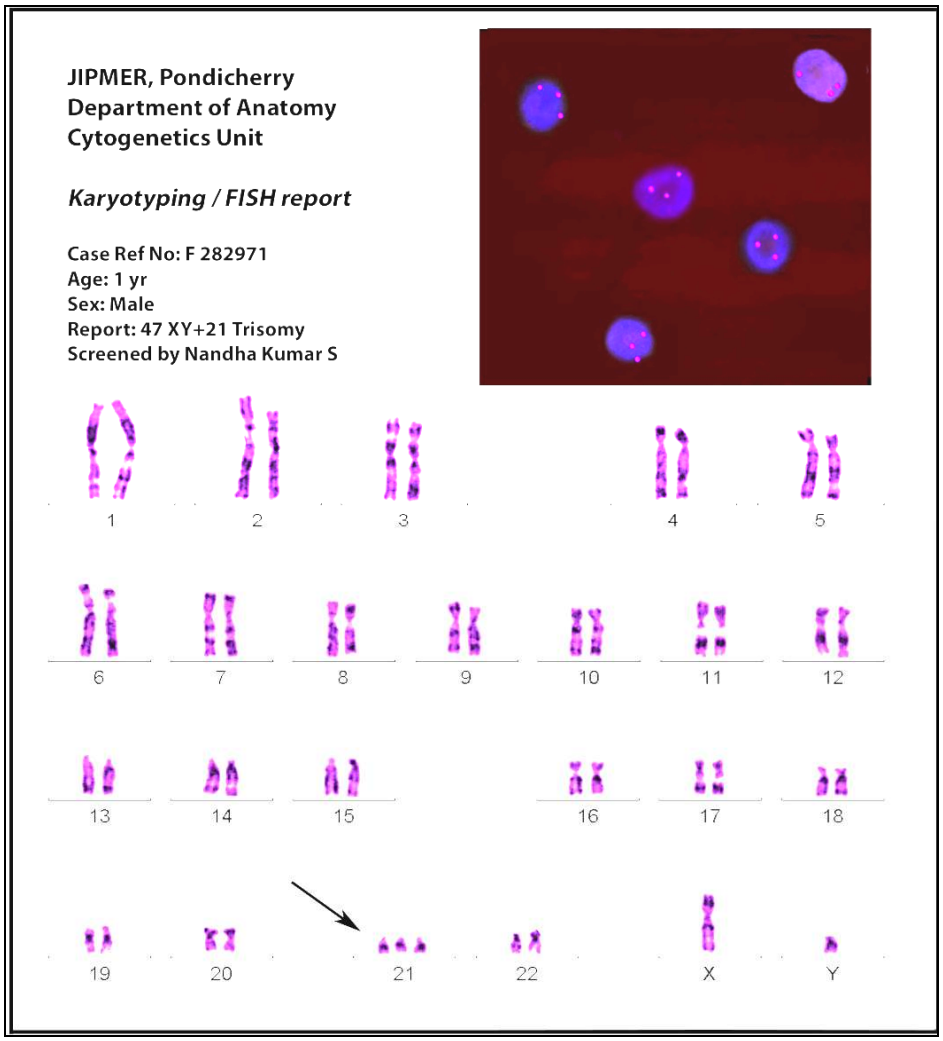


***Figure 1.*** *Karyotype and Interphase FISH reports confirming the clinical diagnosis of Down syndrome.*

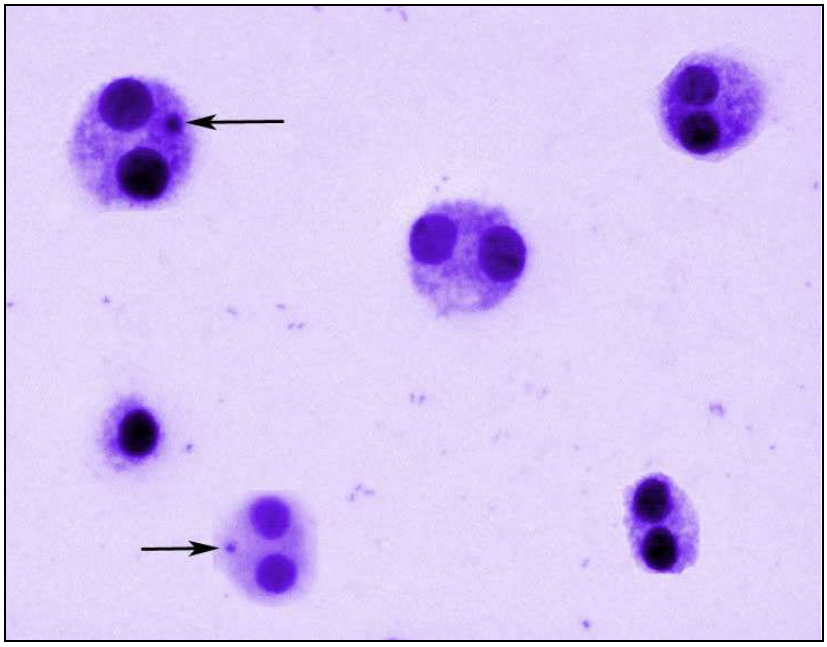

***Figure 2.*** *Arrows indicate binucleated cells with Micronuclei, 40X, Geimsa*

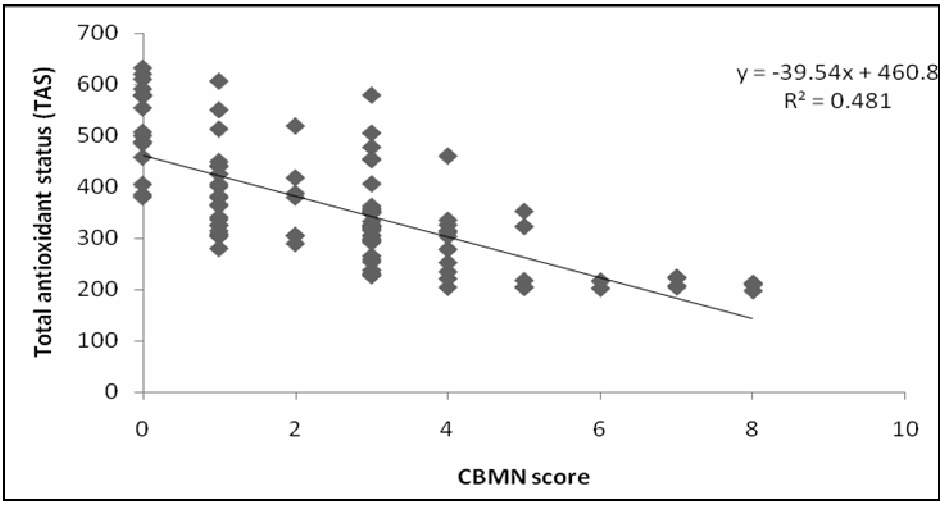


***Figure 3.*** *Correlation between CBMN score and TAS in cases with DS.*

## Discussion

It is well established that increased oxidative stress occurs in DS. Free radicals damage the DNA, cause impairment in its repair capacity and thereby induce chromosome breaks and MN accumulation. Quantification of MN by CBMN assay offers several advantages being simple, sensitive and reliable. Moreover, it does not require the presence of cells in metaphase.

Studies involved in establishing the MN frequency in exfoliated buccal cells from the oral cavity among DS patients have shown a significant increase in MN score compared to that of controls [9,10]. They have also come out with an interesting finding that the MN frequency shows a two fold increase in older DS group compared to younger. Our results are in line with the above finding pertaining to MN frequency between DS cases and controls. But, regarding the increase of MN frequency with age, we could not substantiate it because the DS subjects included in the current study were all of Pediatric age group with a mean ± SD age of 3.03 ± 4.1. Furthermore, CBMN assay performed with additional treatment of Mitomycin C (DNA crosslinker) in DS lymphocytes showed increased sensitivity to the said drug and presented an increased incidence of MN formation compared to con

trols [11-15]. A similar increase of MN frequency with age was observed in studies involving Mitomycin C. This proves that DS genome is highly vulnerable to exogenous genotoxic agents.

Antioxidant mechanism plays an important role in detoxifying the deleterious effects of free radicals and thereby maintains the redox equilibrium. In the said process, it gets depleted if there is excessive generation of free radicals as in the case of DS. Significant low levels of TAS observed in the present study infers increased free radicals production and oxidative stress in DS and also stands out to be the reason for increased CBMN score observed. When CBMN score was compared with TAS in DS cases, a significant negative correlation was observed between them. This result indicates that there is an increase in CBMN score with decrease in TAS.

In conclusion, CBMN score and TAS were significantly increased and decreased respectively in DS cases and both were negatively correlated. Our results suggest that CBMN assay is very sensitive in indicating the genotoxic effects of ROS on the nuclear material by means of MN frequency. Hence, we suggest that CBMN score is a potential biomarker of genotoxicity in cases with trisomy 21.

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