Section: Pathology

Research

EVALUATION OF DNA DAMAGE IN TYPE I DIABETIC PATIENTS BY SISTER CHROMATID EXCHANGE- A CASE CONTROL STUDY.

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Abstract

Background: To assess the DNA damage through Sister Chromatid Exchange (SCE) in Type I Diabetic patients. **Materials and Methods:** In this Case-Control study, 30 clinically diagnosed Type I Diabetic patients confirmed through C-peptide levels was recruited. SCE was carried out using conventional lymphocyte cell culture with 100 µl of 5' Bromo-2'-deoxyuridine and deoxycytidine. At the 67th hour of incubation, the culture was arrested using colchicine and metaphase spreads were stained using Fluorescence plus Giemsa. The SCE switch points were scanned using IKAROS software, Germany. Sister chromatid exchanges in each metaphase spread were calculated for cases and controls. **Result:** The mean ages of the cases were 24.6±6.7 yrs, and controls were 25.9±5.7 yrs. The mean duration of diabetes in the cases was 5.8±4.8 yrs. The sister chromatid exchanges showed a significant difference between the cases and controls. The mean values of SCE/cell of cases (7.79±1.45) were statistically significant compared to controls (4.47±1.12), p-value being <0.0001. **Conclusion:** Sister chromatid exchange was substantially higher in cases than in controls, indicating greater DNA damage.

INTRODUCTION

India has the highest number of diabetic subjects in the world, garnering the dubious title of "diabetes capital of the world.".[1] The large majority of diabetic patients fall into one of two categories: type 1 diabetes mellitus, which is attributed to an absolute or near-absolute insulin deficiency or type 2 diabetes mellitus, which is characterized by insulin resistance and insufficient compensatory insulin secretion.[2] In diabetes, several etiological processes contribute to hyperglycemia, which includes reduced insulin secretion, decreased glucose utilization, and increased glucose production. In a patient with type 1 diabetes, hyperglycemia is caused by genetic, environmental, and immunologic factors, which results in pancreatic beta cell death and insulin deficiency.[3] The metabolic dysregulation causes secondary pathophysiological changes in multiple organ systems. Glucose autooxidation and nonenzymatic glycation of macromolecules produce reactive hydroxyl radicals, resulting in increased oxidative stress. DNA damage caused by oxidative stress appears to have a role in the aetiology of type 1 diabetes and its consequences.[4] Sister chromatid exchanges were first observed by McClintock in 1938 in ring chromosomes of maize. Sister Chromatid Exchange (SCE) analysis is a great sensitive method for assessing DNA damage quantitatively and qualitatively.[5] SCE represents the DNA replication products exchange at seemingly homologous loci. These exchanges are presumed to be because of DNA breakage and reunion.[6]

Aim

To evaluate the DNA damage in type I diabetic patients using sister chromatid exchange analysis.

MATERIALS AND METHODS

Thirty types I diabetic patients with C peptide levels less than 0.5ng/ml from the Out Patient Department of Medicine and the Special Clinics of JIPMER Hospital, Pondicherry, and an equal number of age sex-matched healthy controls formed the material for this study. Both males and females in the age group from 15 to 50 years were included for genomic instability test at Cytogenetic Division, Department of Anatomy and Department of Biochemistry, JIPMER, respectively. The study proposal was placed at Institute Research Council, JIPMER and Ethical Committee JIPMER (vide ref no. i.e. C No. SEC/2011/1/1) and approved. Written informed

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consent was obtained from both the patients and controls. C peptide level was measured using the Chemiluminescence method using autoanalyzer SEIMENS Advia Centaur Cp, and DNA damage was estimated using Sister chromatid exchange assay. Sister chromatid exchange was performed by adding 5-8 drops of heparinized peripheral blood drawn from the cases and controls to bromodeoxyuridine and deoxycytidine. Each vial is labelled and covered with aluminum foils and transferred to an incubator at 37deg Celsius for 67 hours.

At the 67th hour, colchicine was added for metaphyseal arrest. The cultures were further incubated for one hour. At the 68th hour, the cultures were centrifuged, and the supernatant was removed, leaving the cell sediment at the bottom of the test tube. To the cell sediment, 10 ml of prewarmed potassium chloride hypotonic solution was added and further incubated for 45 minutes at 37deg Celsius. Again, centrifugation was repeated, and the cell pellets were separated. Then, the cell pellet was resuspended in a freshly prepared chilled fixative. The cell pellet thus obtained after effective fixation was dropped on a clean cold slide from a height of about one foot. Slides were then dried on a warmer and subjected to staining after 4-7 days. Slides were stained using Fluorescence plus Giemsa method and studied using Olympus BX 51 microscope. Sister chromatid exchange was analyzed using automated Karyotyping software – Ikaros Metasystems, Germany. The data obtained were subjected to statistical analysis using INSTAT Graph Pad software 3.10 and SPSS software to draw the various levels of significance.

RESULTS

Out of the 30 cases, 20 were males, and 10 were females. Out of the 30 Controls, 17 were males, and 13 were females. The cases and controls were further subdivided into two groups with age less than or equal to 25 and age more than 25. Sister chromatid exchange among the cases and controls showed a significant increase of SCE/cell among the cases compared to controls (p-value <0.0001), considered highly significant. Comparing the SCE/cell of cases and controls between the two subgroups showed that the levels are highly significant at the subgroup level also. But there was no significant difference between the subgroup of cases. Structural chromosomal aberrations like chromosomal loss, interchromatin adhesions, chromosomal loss and dicentric chromosomes are also noted in cases than in controls.

Table 1: Distribution of Sister chromatid exchange levels per cell in various age groups of cases and controls

		Cases	Control	P value
SCE/Cell		7.79±1.45	4.47±1.12	<0.0001
Age	≤25	7.71±1.55	4.24±1.20	<0.0001
	>25	7.92±1.33	4.62±1.05	<0.0001

DISCUSSION

Diabetes and pre-diabetes have recently increased dramatically in India, comprising about 17% of the world's diabetes burden. [7,8] Type 1 diabetes is a complex multiorgan autoimmune disorder where both genetic and environmental factors, such as nutrition or infection, act as triggers and result in pancreatic cell damage.[9,10]

In diabetic patients, especially those with poor glycemic control and hypertriglyceridemia, the production of reactive oxygen species (ROS) and lipid peroxidation is elevated. ROS causes damage to cellular macromolecules. Oxidative stress-induced DNA damage appears to have a role in the pathogenesis of type-1 diabetes mellitus (T1DM) and its complications. Some in-vitro tests like Sister Chromatid Exchange (SCE) test, chromosomal aberrations analysis, and the cytokinesis-block micronucleus assay in lymphocytes have been developed to assay chromosomal damage. SCE is a natural process where two chromatids exchange certain homologous sections of DNA sequence. When genotoxic chemicals damage cellular DNA, the rate of sister chromatid exchange increases. [10-12]

This case-control study aimed to analyze and evaluate the sister chromatid exchange in type I diabetic patients.

In the current investigation of Type I Diabetic patients, the sister chromatid exchange score (SCE/cell) was 7.79±1.45 in cases compared to 4.47±1.12 in controls. The data was statistically significant. Also, no significant difference in SCE levels between the two age groups.

Cinkilic et al., in their case-control study, evaluated 35 type-1 diabetic patients and 15 healthy for frequency of sister chromatid exchange as a part of their investigation. T1DM patients displayed a statistically significant and higher frequency of SCE (5.44±1.47) compared to the control (2.54±0.82). They also divided the study subjects into three age groups (<25, 25-44, and ≥ 45 years). Similar to the present study, the cases showed a significantly higher level of SCEs across all the age groups. However, the values of SCE in the current study were slightly higher.[5]

A randomized case-control study of 20, type 2 diabetic patients and 15 controls was carried out by Sheth et al. to assess the frequency of SCEs. The SCE/metaphase in T2Dm was significantly higher with values like the present study, while the values were also higher in control group. Also, the values in people with diabetes were higher across all the age groups studied than in the controls.[13]

The similarity in results is noted with another study of 25 T2DM patients and 15 healthy controls

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evaluated for SCE analysis. A significant difference in SCE/metaphase was noted between T2DM patients (2.25±0.08) and controls (1.28±0.04).[14]
Parallel results were also noted in the study by Nour El Din Abd El-Baky et al. The research group evaluated 51 T1DM cohorts and compared them with 15 healthy individuals to evaluate the occurrence of sister chromatid exchanges. Diabetic children showed a significantly higher frequency of SCE (5.93 ±2.06) than controls (3.92± 1.25).[11]
Coherence in results is also noted in Blasiak et al. study to investigate the frequency of SCE in 50 T2DM patients compared with 30 healthy controls. T2DM patients showed a significantly higher frequency of SCE as compared to controls (7.11 ± 1.14 and 4.96 ± 0.92).[15]
Contradictory results were noted in the study by Vormittag, who found a higher level of SCE in controls compared to T2DM cases, but the difference was not statistically significant.[16]

Limitations

Our study has several limitations, like a smaller number of subjects' lack of evidence to support the increase in sister chromatid exchange in diabetic patients. The scope of the study can be further expanded by studying the oxidative stress level in these patients and correlating it with the DNA damage.

CONCLUSION

The study showed increased DNA damage, which manifests as sister chromatid exchange is significantly elevated in cases than controls. Therefore, the reason for elevated DNA damage in diabetic patients needs to be studied. Alleviating the DNA damage in diabetic patients would reduce further morbidity in them.

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