Analysis of Chromosomal Aberrations and Micronuclei in Type 2 Diabetes Mellitus Patients

Pappuswamy Manikantan, Nanditha Rajesh, Aaggi Maria Philip

Department of Life Sciences, CHRIST (Deemed to be University), Bengaluru, Karnataka, India.

Abstract

Introduction: Type 2 diabetes mellitus is a metabolic disorder characterized by insulin resistance and disrupted insulin secretion. It is often linked to injuries, malfunction and failure of several organs in the long term. The elevated chromosomal disruptions and genetic complications in diabetic patients are due to the increased production of reactive oxygen species. Materials and Methods: The current study used chromosomal aberration assay and micronucleus assay to analyze the extent of abnormalities in the subjects. Results: The results showed increase in frequency of chromosomal aberrations in diabetic patients when compared to the control group (2.76±1.65 and 0.47±0.75 respectively). They also showed higher levels of micronuclei formation than the control participants (13.28±8.63 and 4.12±8.89 respectively). The correlation analysis indicated positive relationship between total aberrations and duration of diabetes. Conclusion: These results indicate that diabetes is associated with genomic instability and studies at a genetic level can be employed for early detection.

Keywords: Chromosomal aberration assay- micronucleus assay- oxidative stress- type 2 diabetes mellitus

Introduction

Diabetes is a rapidly growing epidemic which is affecting several hundreds of people including children and adults. Type 2 diabetes mellitus (T2DM) is a diseased condition in which the blood glucose levels are abnormally high due to insulin resistance and pancreatic β cell dysfunction. According to the International Diabetes Federation (IDF), in 2017 approximately 425 million adults were diagnosed with diabetes and it is estimated that the number will rise to 629 million by 2045. And also diabetes had caused about 4 million deaths in 2017 all over the world (idf.net access).

T2DM is a hyperglycemic condition which is triggered by genetic factors, physical inactivity, sedentary lifestyle and high caloric diet. Unlike type 1 diabetes which affects children and young adults T2DMAffects people of age group 45-64. But nowadays due to different factors T2DM is increasingly being diagnosed at a younger age [4]. Obesity is considered as one of the significant factor that can lead to metabolic disorders such as T2DM [5].

Increased release of non-esterified fatty acids (NEFA) and diacylglycerol have a direct effect on insulin resistance. These molecules activate Protein Kinase C which inhibits the signaling pathway initiated by the binding of insulin receptor and Insulin Receptor for Substrate 1 (IRS-1) thereby leading to insulin resistance [6]. As the disease progresses about 50% of pancreatic β cells get damaged causing decreased insulin secretion [7].

Inflammatory
mediators, mitochondrial dysfunction, reactive oxygen species (ROS) etc. add on to the effect. And also maternal nutrition during pregnancy has an influence on diabetic predisposition of the infant which is well explained by thrifty phenotype hypothesis. The hypothesis states that poor nutrition in fetal and infant life can lead to reduced development and function of β cells and insulin sensitive tissues [8]. All these factors pave way for the development of the disease.

Diabetes not only leads to several metabolic syndromes but can also cause extensive DNA damage. T2DM is often associated with sister chromatid exchanges, chromatid breaks, decreased telomere length and formation of micronuclei [9-10]. The formation of micronucleus is very common in diabetic patients and can be attributed to the abnormality in segregation of chromosomes, presence of lagging acentric chromosomes and chromatid fragments [1]. Oxidative stress is main cause of DNA damage [11]. Due to the hyperglycemic condition and altered metabolisms non-enzymatic glycation of proteins, lipids and nucleic acids occur which in turn result in increased release of highly reactive oxygen free radicals [12]. The reactive oxygen species (ROS) such as O₂⁻, OH⁻, H₂O₂ have the ability to degrade DNA. And also it is seen that diabetic patients often have a reduced antioxidant capacity and impaired DNA repair mechanism [10]. The free radicals produced in the mitochondria cause damage to mitochondrial DNA and result in the dysfunction of the organelle [13]. All these factors lead to chromosomal instability in the patients and can be responsible for further complications. DNA damage is analyzed through genotoxic studies such as cytokinesis-block micronucleus (CBMN) assay, chromosomal aberration (CA) assay, comet assay etc. in lymphocytes and thereby used to predict the chances of occurrence of diabetes in the future. In cases of early prediction, the disease can be prevented by opting a healthy diet, physical activity etc. It has been studied that by adopting a hypocaloric diet the disease can be prevented or even reversed to an extent as when fat content in reduced insulin sensitivity gets normalized [7].

Since there is huge gap between cytogenetics and diseases like diabetes, present study is focused on cytogenetic analysis of T2DM patients of South Indian origin (Erode, Tamil Nadu) using techniques like chromosomal aberration (CA) and micronucleus (MN) assay.

Materials and Methods

Study Population

A total of 34 patients having type 2 diabetes were considered for the study. The patients were within the age group of 37-70 years. A control group of 34 samples was also included in the study. The control subjects had normal blood glucose levels and none had a family history of diabetes. The general characteristics and lifestyle details of the study population were collected with the help of a questionnaire. Informed consent was obtained from all participants and the study was performed in accordance with Declaration of Helsinki and with the approval of local ethics committee.

Lymphocyte cultures and cell harvesting

Peripheral blood samples were drawn by venipuncture and stored in sodium-heparin vacuulators. For each donor two blood cultures were set up. 0.5-ml whole blood sample was added to a culture medium (5ml) containing RPMI 1640 medium (pH 6.8-7.2), 10% fetal calf serum, 10μg/ml phytohemagglutinin L (PHA-L), 0.5 mg/ml l-glutamine, and antibiotics (100IU/ml penicillin, 100μg/ml streptomycin). Incubated for 72 hours at 37°C.

Chromosomal Aberration assay

Colcemid (0.1μg/ml) was added 70 h after initiation of all cultures. The cells were harvested and processed through treatments with a hypotonic solution (0.075M KCl) and fixative (3:1 methanol: glacial acetic acid). The slides were prepared and stained using Giemsa solution (4%) for 3 min. The slides were observed under light microscope at 100X magnification.

Cytokinesis block micronucleus assay

This assay was performed by adding Cytochalasin B (6μg/mL) at the 44th hour of incubation at 37°C. The cells were harvested at the end of 72 h of incubation. The cells were treated with cold (8°C) hypotonic solution (0.075M Potassium chloride) and followed by fixation with methanol: Glacial acetic acid (3:1). The slides were prepared and stained using Giemsa solution (4%) for 3 min.

Statistical analysis

The general characteristics and frequency of CA were expressed in terms of mean and standard deviation. The significance levels of total CA and MN percentage in control and diabetic groups were analyzed by Z-test. The relationship between total CA, duration of diabetes and BMI was determined using Pearson’s correlation test. All statistical analysis was carried out with the help of SPSS 21.0 software. A p value <0.05 was considered to be statistically significant.

Results

The general characteristics of experimental and control subjects are listed in Table 1. The mean ages of the diabetic patients and control participants were 54.3 ± 7.83 and 54.44 ± 9.36 years respectively. The duration of diabetes was found to be 8.62 ± 3.66 years (range: 2-17years). The experimental group showed higher values of BMI (36.47 ± 8.52kg/m²) when compared to the control group (29.82 ± 7.44kg/m²). The frequencies of CA and MN of the study population were expressed as mean ± standard deviation and are listed in Table 2. The patients showed different types of CA such as deletion, translocation and inversion. About 95% of the diabetic patients were found to have more than one aberration. The subjects of age 49 and above had more number of chromosomal anomalies (≥4) when compared to people of other age groups. From the data it was observed that deletion is the major aberration among the experimental subjects.

In the control population, 12 subjects showed one or
Table 1. General Characteristics of Study Population

| S.No | Parameters       | Experimental | Control |
|------|------------------|--------------|---------|
| 1    | No. of individuals | 45           | 45      |
| 2    | Gender           |              |         |
|      | Male             | 24           | 24      |
|      | Female           | 21           | 21      |
| 3    | Age (mean ± SD)  | 54.3±7.83    | 54.44±9.36 |
| 4    | BMI in kg/m² (mean ± SD) | 36.47±8.52 | 29.82±7.44 |
| 5    | Duration of diabetes (mean ± SD) | 8.62±3.66 | -       |

Table 2. Frequencies of CA and MN in Study Participants

| Groups       | Deletion (mean ± SD†) | Translocation (mean ± SD†) | Inversion (mean ± SD†) | Total (mean ± SD†) | MN‡ /1000 cells (mean ± SD) |
|--------------|-----------------------|---------------------------|------------------------|-------------------|--------------------------|
| Experimental | 1.41±1.30             | 0.91±0.79                 | 0.44±0.56              | 2.76±1.65*        | 13.28±8.63*              |
| Control      | 0.15±0.36             | 0.18±0.46                 | 0.15±0.43              | 0.47±0.75         | 4.12±8.89                |

†SD- standard deviation; ‡MN- micronucleus * values are significant at p<0.05 when compared to control samples.

the other anomalies. Translocation was the frequently observed type of aberration among the control samples. When compared to control population, experimental subjects showed higher rates of chromosomal damage. The total frequency of chromosomal anomalies of experimental group was found to be 2.76 ± 1.65 which is significantly higher than the control group whose frequency was 0.47 ± 0.75. This was proved statistically using Z-test. The frequencies of deletion, translocation and inversion in the experimental group were found to be 1.41 ± 1.30, 0.91 ± 0.79 and 0.44 ± 0.56 respectively. Meanwhile, the control group showed the frequencies to be 0.15 ± 0.36, 0.18 ± 0.46 and 0.15 ± 0.43 respectively. The percentage of micronuclei in experimental participants was observed to be 13.28±8.63 and that of control group was 4.12 ± 8.89. The MN percentage of the diabetic group was also significantly higher than the control group which was statistically established using Z – test. When analyzed through Pearson’s correlation test total CA and duration of diabetes showed moderate positive correlation though there was no significant relationship between total CA and BMI.

Discussion

Type 2 diabetes which is a hyperglycemic condition is associated with genetic and chromosomal variations. The abnormalities can be attributed to increased release of highly reactive oxygen free radicals [12]. Increased release of interleukins can lead to ROS production which indeed cause insulin resistance and β- cell dysfunction which are characteristics of T2DM. The hyperglycemic state results in glycation of proteins and auto-oxidation of glucose which augments the formation of ROS [14]. Genotoxicity studies such as chromosomal aberration (CA) assay and micronucleus (MN) assay are common methods to analyze the anomalies. There are several studies establishing chromosomal damages in T2DM patients. From the present study it was found that diabetic patients have significant chromosomal aberrations when compared to the control group. Boehm et al (2008) showed that T2DM women carry stable chromosomal aberrations when compared to healthy subjects [9]. They suggested that these stable oddities can be a senescence marker of premature death in diabetic women. Body Mass Index (BMI) has strong association with diabetes. Obesity leading to insulin resistance and impaired insulin secretion can be a major cause of T2DM [5]. Overweight individuals are at higher risk to have DNA damage due to oxidative stress [1]. In the study it was observed that the experimental group had higher BMI values than control subjects. But there was no significant correlation between total CA and BMI in the experimental group. The formation of micronucleus is another parameter which determines the extent of DNA abnormalities. According to Corbi et al., (2014) reported elevated micronucleus formation in diabetic patients [1]. These people have high number of binucleated cells with micronuclei and nucleoplasmic bridges. Shettigar et.al., (2012) observed modest increase in MN frequency in poorly controlled diabetic patients [12]. According to the study conducted by Bronson et.al.,(2015) frequency of MN increases with duration of diabetes in the experimental samples [11]. The results of the current study are also in accordance with the previous studies and shows increased MN frequency in diabetic group. Nevertheless, few studies showed no linkage between diabetes and increased DNA damage and this can be due to duration of the disease in the patients considered for the particular study [15]. As per the study by [16] it the percentage of sister chromatid exchange (SCE), duration of diabetes and age in type-1 mellitus patients showed no significant correlation though the present study reported a moderate positive correlation between total CA and duration of diabetes.

Genotoxic studies are very much important in diseases like T2DM which causes psychological and physical stress to patients and put a huge burden on health-care systems. The extent of DNA damage increases the chances of
microvascular and macrovascular complications such as dyslipidemia, periodontitis, cardiovascular diseases and neuropathy which are often associated with diabetes. The chromosomal anomalies are the result of oxidative damage caused by ROS. Oxidative stress over time can cause structural and functional changes in chromosomes. This can often cause diseased conditions like cancer [16]. As the primary objective of any treatment strategy is to slow down the progression of metabolic deterioration, it is important to establish methods for early disease identification and disease management. This can be done by conducting further cytogenetic studies with a large sample size in order to analyze the genetic conditions in diabetic patients.

Acknowledgements

We would like to thank Fr. Joby Xavier, Head of the Department of Lifesciences, Christ (Deemed to be University), Bangalore, Karnataka, India for providing us the with the opportunity and requirements needed for the accomplishment of the research project. We extend our gratitude to Dr. R. Chandirasekar, Faculty, Sri Vasavi College (Affiliated to Bharathiar University), Erode, Tamil Nadu, India for supporting us with the technical help needed in the cytogenetic work. We also appreciate all study participants for volunteering to partake in the study.

References

1. Corbi SC, Bastos AS, Ottico SR, Secolin R, Dos Santos RA, Takahashi CS, et al. Elevated micronucleus frequency in patients with type 2 diabetes, dyslipidemia and periodontitis. Mutagenesis. 2014;29(6):433-9.
2. Hills AP, Arena R, Khunti K, Yajnik CS, Jayawardena R, Henry CJ, et al. Epidemiology and determinants of type 2 diabetes in south Asia. The lancet Diabetes & endocrinology. 2018;6(12):966-78.
3. Jayawardena R, Ranasinghe P, Byrne NM, Soares MJ, Katulanda P, Hills AP. Prevalence and trends of the diabetes epidemic in South Asia: a systematic review and meta-analysis. BMC public health. 2012;12:380.
4. Chatterjee S, Khunti K, Davies MJ. Type 2 diabetes. Lancet (London, England). 2017;389(10085):2239-51.
5. Al-Goblan AS, Al-Alfi MA, Khan MZ. Mechanism linking diabetes mellitus and obesity. Diabetes, metabolic syndrome and obesity: targets and therapy. 2014;7:587-91.
6. Goldstein BJ. Insulin resistance as the core defect in type 2 diabetes mellitus. The American journal of cardiology. 2002;90(5a):3g-10g.
7. Taylor R. Type 2 diabetes: etiology and reversibility. Diabetes care. 2013;36(4):1047-55.
8. Praveen PA, Kumar SR, Tandon N (2015). Type 2 diabetes in youth in South Asia. Curr Diab Rep.15(2):571-574.
9. Boehm BO, Moller P, Hogel J, Winkelmann BR, Renner W, Rosinger S, et al. Lymphocytes of type 2 diabetic women carry a high load of stable chromosomal aberrations: a novel risk factor for disease-related early death. Diabetes. 2008;57(11):2950-7.
10. Haleem A M HSM. Cytogenetic and Immunogenetic Study of Type -2 Diabetes Mellitus Patients. International Journal of Diabetes Research. 2013;2(4):76-80.