Cytogenetic study of type 2 diabetes mellitus

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Abstract—Type 2 of diabetic mellitus (DM) is characterized by chronic hyperglycemia with abnormalities of fat and protein metabolism resulting from defects in insulin secretion, insulin action or both. To understand cytogenetic changes associated with type 2 diabetic mellitus, cytogenetic analysis was done on peripheral blood lymphocytes collected from twenty five type 2 DM patients using short term lymphocyte culture, and 15 apparently healthy people with as control group. The present study includes evaluation the cell cycle changes by determining blast and mitotic indices (BI and MI), as well as study the cellular kinetics by scoring replicative index (RI). The present data revealed significant depressions in BI [41.69±2.28] in patient's group compare with healthy group [54.46±4.56] and in MI [0.52±0.04] compare with healthy group [1.2±0.11] (P<0.01). The rate of cell cycle progression was also a reduction in diabetic patients [1.44± 0.10] in comparison with control [1.88± 0.01](P<0.05). We conclude that cytogenetic indices (BI, MI, and RI) are sensitive tools to achieve the cytogenetic abnormalities in type-2 diabetic mellitus.

Keywords—paper publishing, journals, styles, how-to

1 Introduction

Diabetes Mellitus (DM) is a metabolic defects of multiple etiologies characterized by disturbances of protein, fat and carbohydrate metabolism resulting from disorders in insulin secretion, insulin action or both (1,2). It is a major worldwide health problem predisposing to markedly increased cardiovascular mortality and serious morbidity and mortality(3,4). The prevalence of diabetes mellitus is increasing globally, by 2030, the number of diabetes is expected to increase to 366 million (5,6). Cytogenetic analyses of peripheral blood lymphocytes cultures is sensitive tool in a wide range of studies designed to enquire the genetic effects of environmental agents. Human blood of studies designed to enquire the genetic effects of environmental agents. Human blood lymphocytes have long been utilized to assess genetic damage occurring in vivo and in vitro. The most common kinds of chromosomal aberrations and sister chromatid exchanges (SCEs) are tightly related to other genetic changes such as mutations (7), and
neoplastic transformation (8). Other test systems that have been used in cytogenetic analysis including blastogenic index (BI), Mitotic Index (MI), Replicative Index (RI), and sister chromatid exchanges (SCEs) (9). To understand cytogenetic changes associated with type 2 diabetic mellitus, cytogenetic analysis was done on peripheral blood lymphocytes taken from twenty five type 2 DM patients using short term lymphocyte culture.

2 Materials and Methods

Patients whom chosen for this study were men and women with DM disease. They have been chosen from various hospitals in wasit province which include: Al-Zahraa Hospital, Al- Karama Hospital and Wasit Blood Bank. 25 patients with T2DM from two genders (males & females) with ages 30-80 years (mean age: 45.3 years). Fifteen apparently healthy control groups similar to type 2 diabetes mellitus patients in age and gender were comprised in this study. Each patients and control groups were drawn about 2 ml of blood and putting into sterile vacutainer tube (10ml) containing 0.1ml heparin solution and was used for short-term cultures. Short-term peripheral blood lymphocyte cultures RPMI-1640 were done under optimal conditions (10).

Cytogenetic analysis of culturing peripheral blood lymphocytes was done using blood specimens obtained from healthy individuals. In these experiments, various concentrations of colcemide, PHA, in addition to various specimens of blood cultured were used to detect the optimization of the culture media and to obtain clear results. In this study, the standard blood culturing involved inoculation of 0.1ml of heparinized peripheral blood in 3ml of RPMI-1640 culture medium which was manipulated in 10ml sterile vacutainer tube. Adding 0.2ml of PHA, then, incubated the culture medium at 37 °C for 72 hours (11).

3 Cell harvesting method

Colcemide(0.1 ml) in a concentration of 10μg/ml was added to each culture tube for the final two hours of incubation time to harvest of mitogen stimulated cells (15). In the last of incubation time, tubes containing cells were centrifuged at 2000 rpm for 10 minutes. The supernatant was discarded and a little medium was remained over the cell pellet. 8ml of warmed hypotonic solution (37°C) was treated the harvested cells with gentle mixing, then, the tubes were incubated in a water bath at 37°C for 25 minutes with shaking every 5 minutes (11).

The cells were centrifuged at 2000 rpm for 10 minutes, the supernatant was discarded, and the cells were fixed with 5ml of fixative solution which was added as drop-wise with well mixing. Washing the cells at least three times with the fixative solution. Eventually, the cells were suspended in a small volume of the fixative solution and kept at 4°C until slide preparation (15). The slides were examined by light microscope to detect blast index (BI) and mitotic index (MI). The technique of fluorescence plus giemsa (FPG) staining was used to stain 5 slides for each samples for detection of cell
replication kinetics. The FPG modified from a procedure which was reposed from Benn and Perle, (12).

4 Results and Discussion

It is now well certain that the cytogenetic analysis of peripheral blood lymphocytes cultures constitutes an essential and sensitive tool in a wide range of studies designed to achieve the genetic effects in several diabetes. Short-term culture of PHA simulated human lymphocyte is widely utilized to detect chromosome damaging, human exposure to mutagens or carcinogens and the immune response of blood (13). Cytogenetic results demonstrated a significant decrease in blast index (BI) \[41.69±2.28 \ (P < 0.01)\] and mitotic index (MI) \[0.52±0.04 \ (P< 0.01)\] in diabetic patients as compared to these values of control group \[54.46±4.56, 1.2±0.11\] respectively(Table1). The decreasing in BI and MI was obvious in diabetic mellitus which leads to alterations in their cell cycles. PHA are a mitogen which activated T-lymphocytes from G0 to G1 (14).This activated is accompany by profound metabolic modification (15) which results in T-lymphocyte surface acquisition of interleukin-2 receptor (14). According to PHA lymphocytes stimulation mechanism, the decrease in BI may be returned to the disease itself by producing different factors that can repress metabolic pathways related to mitogen receptors (16).

The decrease in MI may be related to diabetic cells are potent factors causing chromosome instability that lead to breakage of the chromosome, and eventually any cell loaded with such chromosomal aberrations arrests in S-phase (17). These cytogenetically visible changes indicates that the alter in the genetic stability of diabetic cells. This result agreement with cytogenetic studies in cancer patients (13, 18).

On the other hand, the results indicated significant decrease in the replicative index (RI) \[1.44±0.10 \ (P< 0.05)\] as compare with control group \[1.88±0.01\] (Table 1). Low replicative index refers that patients with T2D have a low progression of cell cycle. The cell cycle proliferation orchestrated by the activity of serine threonine kinases cyclin D1family which encodes the labile regulatory subunit of the cyclincdk 4/6 holoenzyme (19). The cyclinD1 protein plays crucial role in the regulation of the cell cycle (20). CyclinD1 is a regulator of the cell cycle that promotes the transition from G1 to S phase by activating cyclin-dependent kinase 4(CDK4) or CDK6. Although the functions of cyclinD1 in livers has mostly been studied in the context of liver damage, regeneration, or carcinogenesis (21). Bhallae et al recently revealed that cyclinD1 represses hepatic gluconeogenesis (22), thus cyclinD1 may lead to chromosomal instability.

<table>
<thead>
<tr>
<th>Cytogenetic parameters</th>
<th>Control</th>
<th>Type 2 DM</th>
<th>P-value</th>
</tr>
</thead>
</table>

Table 1. Cytogenetic analyses of peripheral blood lymphocytes of Type 2 diabetic mellitus patients and control group
<table>
<thead>
<tr>
<th>Blast index (BI)</th>
<th>54.46±4.56</th>
<th>41.69±2.28</th>
<th>0.01**</th>
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</thead>
<tbody>
<tr>
<td>Mitotic index (MI)</td>
<td>1.2±0.11</td>
<td>0.52±0.04</td>
<td>0.01**</td>
</tr>
<tr>
<td>Replicative index (RI)</td>
<td>1.88±0.01</td>
<td>1.44±0.10</td>
<td>0.05*</td>
</tr>
</tbody>
</table>

We conclude that Cytogenetic indices (BI, MI, and RI) are sensitive tools to achieve the cytogenetic abnormalities in diabetic mellitus.

5 References