ABSTRACT

The major cause of death in the European Union EU are cardiovascular illnesses. They cover a wide range of medical issues affecting the circulatory system (heart, blood vessels and arteries), often atherosclerosis caused by the abnormal plaque build-up, consisting of cholesterol or fatty substances placed inside the walls of the artery of a person. Some of the most prevalent diseases that affect the circulatory system include ischaemic heart disease (heart attacks) and cerebrovascular diseases (strokes). Fifty Cardiovascular subjects (23 females and 27 males) were enrolled. In addition, thirty healthy control subjects (13 females and 17 males). This study is designed to measure the serum level of interleukin IL-33, Troponin and Single nucleotide polymorphism. The concentration of IL-33 and Troponin were successfully obtained by using enzyme-linked immune sorbent assay (ELISA). While, the single nucleotide polymorphism (SNP) is done by conventional polymerase chain reaction (PCR) and sequencing techniques. Results showed that the serum level of IL-33 was statistically higher in patients than controls, while Troponin levels was not statistically significant. In addition, the SNP was not statistically significant in Iraqi patients.

Keywords: Cardiovascular disease, Interleukin-33, Troponin, Single nucleotide polymorphism, polymerase chain reaction, Sequencing

INTRODUCTION

CVD is still the world's largest cause of death, Global Disease Burden (GBD) Studies 2013 estimate that 17.3 million global deaths were caused by CVD. It accounted for 31.5% of all deaths and 45% of all non-communicable disease deaths, more than twice as many as communicable, maternal, neonatal and dietary disorders have been associated with cancer[1]. In the last three or four decades, aggregated mortality rates ascribed to CVD in developed countries have declined continuously and significantly, perhaps creating some satisfaction that the threat is ended. However, the decrease in the rates of crude mortality has deteriorated in latest years since aging society and increasing obesity and diabetes are compensating for the gains achieved in other fields[2]. Repeated population surveys show noticeable deficiencies in treatment for common CVD danger issues such as high cholesterol and hypertension[3-5]. IL-33 is a new member of IL-1 who controls...
innate and adaptive immune systems to encourage inflammatory reactions\textsuperscript{[6]}.
Keratinocytes, epithelial and endothelial cells are the main expressions of IL-33.\textsuperscript{[7]} In addition to human monocytes\textsuperscript{[8]}, IL-33 activates numerous immune cell kinds convoluted in type-2 immunity and allergic inflammation, including ILC2s, mast cells, Th2 cells, eosinophils, basophils, dendritic cells and alternatively activated macrophages (AAM)\textsuperscript{[9-11]}. In the nucleus of the production cells, IL-33 accumulates\textsuperscript{[12-14]}, binds to histones and chromatin\textsuperscript{[13,15]}. Furthermore, the development of the N-terminal nuclear domain promotes a crucial function in nuclear localization and the chromatin-association IL-33. Initial studies indicated that IL-33 displays certain transcriptional repressor properties when overexpressed in mammalian cells\textsuperscript{[13,16]}. Later more than 10 years of investigation efforts, convincing evidence that endogenous nuclear IL-33 regulates gene or protein expression is still missing. We have lately shown that extracellular IL-33 cytokine, rather than nuclear endogenous, regulates protein expression in main human endothelial cells through a worldwide proteomic strategy\textsuperscript{[17]}.

Troponin is a striated muscle protein complex that is inhibitory or contractile. It is regularly situated along the muscle's thin filament and consists of three different proteins: troponin I, troponin C and troponin T\textsuperscript{[18-22]}. Troponin I (TnI) contains three isoforms. Two are existing in skeletal muscle and the other is present only in cardiac muscle. With a molecular weight of 24,000 Da, the cardiac isoform (cTnI) has a greater weight than the other isoforms since it contains an additional 32 amino acids. The other protein is more than 40% different from the skeletal muscle TnI in its amino acid sequence\textsuperscript{[23-25]}.

MATERIALS AND METHODS

Sample collection

The samples composed of fifty CVD patients (27 male and 23 female) and thirty genetically unrelated, healthy volunteers (17 men and 13 women). The blood samples collection extended through the period of October 2018 to February 2019. Blood samples of patients were collected from Ibn Al-Bitar Specialized Center for Cardiac Surgery, Baghdad- Iraq. All patients gave their informed written approval to participate in the study. The diagnosis was confirmed by experienced doctors. The entire participants were of unrelated Iraqi origin and had similar geographic data.

Blood sample collection

A blood sample was collected from both CVD patients and healthy control by using 10 ml of disposal syringe in gel-containing tubes, left to clot at room temperature (20-25 °C) for 10 minutes, then centrifuged at 5000 rpm for 5 minutes to obtain serum. Serum was separated after centrifugation and divided into three Eppendorf tubes to avoid multiple freezing and thawing and kept frozen for further.

Laboratory methods

Serum level of IL-33 and Troponin was determined by Using Enzyme-linked immunosorbent assay (ELISA). DNA was extracted from blood leucocytes using ReliaPrep\textsuperscript{TM} Blood g DNA Miniprep kit (promeg/USA) according to the manufactures instructions. Polymorphism rs7025417 was assessed by PCR and sequencing techniques. Primer sequences was designed by Macrogene Company (Korea).

Statistical analysis
Analysis of data was carried out by using the available statistical package of SPSS-25 (Statistical Packages for Social Sciences- version 25). Data were presented in simple measures of frequency, percentage, mean, standard deviation, and range (minimum-maximum values). The significance of the difference of different means (quantitative data) was tested by using Students t-test for the difference between two independent means and Pearson correlation was calculated for the correlation between two quantitative variables with its t-test for testing the significance of correlation.

RESULTS AND DISCUSSION

Serum level of IL-33

The serum level of IL-33 was significantly highly increased (P=0.004) in patients experiencing CVD compared to controls 115.33±116.20 versus 59.72±52.53 pg/ml, as shown in Table 1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>No.</th>
<th>IL-33 concentration (pg/ml)</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVD</td>
<td>50</td>
<td>115.33±116.20</td>
<td>0.004*</td>
</tr>
<tr>
<td>Control</td>
<td>30</td>
<td>59.72±52.53</td>
<td></td>
</tr>
</tbody>
</table>

*Significant difference P< 0.05

Serum level of Troponin

Table 2 shows the mean distribution of Troponin in CVD patients compared to healthy controls. The results did no show relative change in the level of Troponin in CVD patients compared to control 6.83±1.88 versus 6.30±0.50 mg/ml the difference was not statistically significant (P= 0.136).

<table>
<thead>
<tr>
<th>Groups</th>
<th>No.</th>
<th>Troponin concentration (mg/ml)</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVD</td>
<td>50</td>
<td>6.83±1.88</td>
<td>0.136 NS</td>
</tr>
<tr>
<td>Controls</td>
<td>30</td>
<td>6.30±0.50</td>
<td></td>
</tr>
</tbody>
</table>

NS = non-significant P > 0.05

SNP (rs7025417)

Table (3) shows number and percentage of IL-33 gene genotype in CVD patients and controls group

<table>
<thead>
<tr>
<th>Genotype</th>
<th>CVD(n=50)</th>
<th>Controls(n=30)</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>TT</td>
<td>14</td>
<td>70.0</td>
<td>6</td>
</tr>
</tbody>
</table>

DISCUSSION

In the current study, we measured the concentrations of IL-33, Troponin, and investigated the gene polymorphism in eighty individuals of Iraqi origin, including fifty CVD patients and thirty appear healthy controls.

Results showed a higher level of IL-33 was encountered in CVD patients, nuclear IL-33 is released from necrotic human coronary artery smooth muscle cells, human adult cardiac myocytes and cardiac fibroblasts in vitro therefore cell damage induce release of IL-33[26]. IL-33-ST2L pathway appears to have a causal role in the development of CVD[27], and a high serum concentration of IL-33 was observed in IHD[28]. Troponin can be released into the bloodstream as a result of myocardial injury.[29] reported that Troponin plays a vital role in the diagnosis of Myocardial Infarctions, it is imperative to keep in mind the multiple non-ischemic and non-cardiac causes and potential factors that may lead to elevations of troponin levels.[30] found that Higher hsTnI levels are associated with the underlying burden of coronary atherosclerosis, more rapid progression of CVD, and a higher risk of mortality and incident cardiovascular events. Cardiac troponin concentration within the normal range is associated with increased CVD risk[31]. The result of SNP in Iraqi patient was not significant, while in Chinese population was significant[32].

CONCLUSION

In summary, it was found that IL-33 was associated with CVD, while Troponin levels were not statistically significant. Moreover, the SNP (rs7025417) was not significant, taking into consideration the limitations of the present study. Further studies are needed with more samples.

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ETHICAL CLEARANCE

The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.
FUNDING: Self-funding

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