The correlation between oral warfarin intake and two pro-inflammatory cytokines (IL-6 and TNF-α) and their effects on atherosclerosis in deep venous thrombosis disease.

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Abstract

Warfarin is a commonly used medication for the prevention and treatment of deep venous thrombosis disease. The purpose of this study was to identify the association between warfarin dose and pro-inflammatory cytokines production during the treatment and the effect of this interaction on the development and progression of atherosclerosis in patients with deep venous thrombosis disease. The study was carried out in Al-Sadr Medical City in Al- Najaf province, during the period from Apr, to Dec, 2016. The study involved (70) patients with deep venous thrombosis disease who attended the hospital. The majority of the studies were men (71.4 %) and the most effected age group was from 65 to 74 years old in percentage (44%) followed by the age group 55 to 64 years old in percentage (32%), also the present study showed a highly significant increase mean serum level of IL-6 (464.1 vs. 183.1 pg /ml) and TNF-α (479.2 vs. 191.3 pg/ml) in patients as compared to the corresponding controls, in addition, the results showed that the highly significant increase in both cytokines were in warfarin dose 10 mg/day followed by dose 7.5 and 5 mg/day. It was concluded that warfarin in high doses may have a critical role in the initiation and development of atherosclerosis through its effect on pro-inflammatory cytokines production. It was recommended to achieve a comprehensive investigation for evaluating the side effect of warfarin in high doses on inflammatory process that cause another damage of the immune system leading to atherosclerosis.

Keywords: warfarin, IL-6, TNF-α, atherosclerosis, deep venous thrombosis disease.

Introduction

Deep venous thrombosis disease (DVT) is the formation of blood clot (thrombus) within a deep vein [1], causing occlusion of these veins [2] leading to life threatening especially in massive pulmonary embolism [3]. The beginning of thrombosis is thought to be caused by tissue factor, which leads to conversion of prothrombin to thrombin, followed by fibrin deposition [4] which play a role in attachment of endothelium of blood vessel wall, a surface that normally acts to prevent clotting. Red blood cells and fibrin are the main components of thrombi in veins, another components of thrombi are platelets [5] in addition to white blood cells which effect on the formation of venous clots [6]. Typical symptoms of deep venous thrombosis disease include pain or tenderness, swelling, warmth, redness or discoloration, although these symptoms alone are not sufficiently to make a diagnosis[7].

Atherosclerosis is a disease mainly caused by secretion of several cytokines such as IL-1, IL-6, TNF-α and IFN-γ [8], as well as the presence of large numbers of macrophages, NKT cells and activated CD4⁺⁰T cells within atherosclerotic plaques [9]. IL-6 and TNF-α are cytokines principally produced by monocytes and play important role in
inflammation [10] related to blood coagulation hence, have a large effect on the pathogenesis of atherosclerosis [11]. IL-6 is a cytokine which regulates both humoral and cellular responses and effect on their cytokines production [12]. TNF-α is an early mediator involved in the production of IL-6, chemokines and CRP as well as the recruitment of leucocytes during inflammatory reactions [13]. TNF-α also play a role in lipid metabolism by stimulating liver production of triglycerides and decreasing the activity of lipoprotein lipase and 7-α hydroxylase in addition TNF-α induce smooth muscle proliferation and increase adhesion of leucocytes to endothelial cells [14].

Warfarin is an anticoagulant giving to patients orally and it is fully bounded to albumin in the blood [15] used in the prevention and treatment of thrombosis [16], originally discovered in spoiled sweet derivative of dicoumarol, a 4-hydroxycoumarin-derived mycotoxin. Dosing of warfarin is complicated depending on the interaction with many commonly used drugs and foods, although the mechanism of this interactions are unclear [17] close monitoring for the degree of anticoagulation is done by a blood test measuring an INR (the international normalized ratio) or PT (prothrombin time).

Amis of the study.

This study aimed to determine the effect of warfarin dose therapy on the stimulation of two pro-inflammatory cytokines including IL-6 and TNF-α and to correlate the relationship between this interaction of warfarin and two cytokines IL-6 and TNF-α in the inflammatory process leading to initiation and progression of atherosclerosis.

Material and Method

The cross-sectional descriptive approach was designed to meet the previously reported objectives of this study which was conducted from Apr. to Dec. 2016. In AL. Najaf City / Al-Sadr Medical City . A convenience sample of (70) patients with deep venous thrombosis disease treated with oral warfarin therapy , in addition to (30) samples of apparently healthy individuals with no history of deep venous thrombosis disease or atherosclerosis as control group for comparison with patients was taken in this study.

Data collection:

Data were collected by interview technique with patients with deep venous thrombosis disease on warfarin treatment depending on the diagnosis made by the consultant medical staff at the hospital based on clinical examination and other confirmatory investigations such as ultra sound, a D-dimer and wells score

Cytokine tests:

The level of serum Cytokines including IL-6 and TNF-α were measured using Enzyme linked immune sorbent assay (ELISA) methods (according to the kits from Bio-Source, Europe S.A.)

Calculation of the results:

The sample results were calculated by interplotation from a standard curve using a curve fit equation for both IL-6 and TNF-α.

Statistical Analysis
The values of these parameters were presented as mean ± standard deviation (S.D.), and significant differences between means were assessed by ANOVA test, the least significant difference (LSD) or Duncan’s test by using the computer programme social package for statistical analysis (SPSS) version 7.5 in which a probability (P) equals or less than 0.05 was considered significant.

Results

From the total of seventy patients with deep venous thrombosis disease were included in this study, 25 patients without atherosclerosis and 45 patients have atherosclerosis disease. Moreover male patients showed increased percentage frequencies when compared with female patients (71.4% vs. 28.6%) (table 1).

Table 1: Gender distribution of deep venous thrombosis disease patients and controls.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total number</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Controls</td>
<td>30</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>Patients</td>
<td>70</td>
<td>50</td>
<td>20</td>
</tr>
<tr>
<td>Patients without atherosclerosis</td>
<td>25</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>Patients with atherosclerosis</td>
<td>45</td>
<td>35</td>
<td>10</td>
</tr>
</tbody>
</table>

The distribution of study samples according to age groups showed that the most affected group was between 65 to 74 years old in percentage (42.9%) followed by the age group 55 to 64 years old (31.4%) (table 2).

Table 2: Age distribution of deep venous thrombosis disease patients and controls.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total number</th>
<th>Age group (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>35-44</td>
</tr>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Controls</td>
<td>30</td>
<td>2</td>
</tr>
</tbody>
</table>

URL: http://www.uokufa.edu.iq/journals/index.php/ajb/index
http://iasj.net/iasj?func=issues&jId=129&uiLanguage=en
Email: biomgzn.sci@uokufa.edu.iq
Table 3 showed a highly significant increased mean serum level of IL-6 in patients as compared to control subjects (464.1 vs. 183.1 pg/ml), and the highly increased in mean serum level in warfarin dose 10 mg/day followed by dose 7.5 and then 5 doses.

Table 3: serum level of IL-6 in deep venous thrombosis disease patients divided by doses of oral warfarin intake (mg/day).

<table>
<thead>
<tr>
<th>Warfarin dose (mg/day)</th>
<th>Number</th>
<th>Mean ± S.E. (pg/ml)</th>
<th>P value ≤</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (0) dose</td>
<td>30</td>
<td>183.1 ± 12.2</td>
<td>N.S.</td>
</tr>
<tr>
<td>Total patients (all doses)</td>
<td>70</td>
<td>464.1 ± 14.2</td>
<td>0.01</td>
</tr>
<tr>
<td>2.5 dose</td>
<td>30</td>
<td>236.8 ± 18.7</td>
<td>N.S.</td>
</tr>
<tr>
<td>5 dose</td>
<td>20</td>
<td>488.9 ± 13.2</td>
<td>0.01</td>
</tr>
<tr>
<td>7.5 dose</td>
<td>10</td>
<td>570.9 ± 11.3</td>
<td>0.01</td>
</tr>
<tr>
<td>10 dose</td>
<td>10</td>
<td>600.4 ± 10.1</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Significant difference (P value ≤ 0.05) as compared to the corresponding controls.

N.S.: not significant (P value > 0.05).

Total patients also showed highly significant increased mean serum level of TNF-α as compared to the controls (479.2 vs. 191.3 pg/ml), and the highly significant increased were in warfarin dose 10 mg/day followed by dose 7.5 and 5 mg/day (table 4).

Table 4: serum level of TNF-α in deep venous thrombosis disease patients divided by doses of oral warfarin intake (mg/day).

<table>
<thead>
<tr>
<th>Warfarin dose (mg/day)</th>
<th>Number</th>
<th>Mean ± S.E. (pg/ml)</th>
<th>P value ≤</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (0) dose</td>
<td>30</td>
<td>191.3 ± 14.4</td>
<td>N.S.</td>
</tr>
<tr>
<td>Total patients (all doses)</td>
<td>70</td>
<td>479.2 ± 20.2</td>
<td>0.01</td>
</tr>
<tr>
<td>Dose</td>
<td>N (n)</td>
<td>Value</td>
<td>p-value</td>
</tr>
<tr>
<td>-------</td>
<td>-------</td>
<td>---------------</td>
<td>---------</td>
</tr>
<tr>
<td>2.5</td>
<td>30</td>
<td>251.6 ± 16.1</td>
<td>N.S.</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>450.7 ± 17.3</td>
<td>0.01</td>
</tr>
<tr>
<td>7.5</td>
<td>10</td>
<td>590.6 ± 14.2</td>
<td>0.01</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>660.7 ± 13.4</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Significant difference (P value ≤ 0.05) as compared to the corresponding controls.

N.S.: not significant (P value > 0.05).

Discussion

The importance of this study come from many epidemiological studies which reported that the prevalence of atherosclerosis disease is increasing all over the world [18], these results seems to agreement with the current study results, in which it was found increasing percentage of the atherosclerosis disease among our patients in AL Najaf city as a complication of deep venous thrombosis disease which is a major cause of morbidity and mortality [19], this results supported by another reports in the United States, which is estimated that one from 1000 adults per year has DVT [20]. Up to our knowledge, this is the first study at least in Iraq which is trying to correlate between atherosclerosis and warfarin therapy for treated of deep venous thrombosis disease patients, because the accurate choosing of ideal drug with less side effect remains one of the more challenging areas of medicine. The present study demonstrated that the treatment with warfarin in high doses can stimulate the immune system by increasing the production of two cytokine levels including IL-6 and TNF-α which were considered as inflammatory biomarkers [21] related to blood coagulation [22], the important of these results come from the fact that inflammation is central at all stages of atherosclerosis [23], by playing critical role in initiation and progression of atherosclerosis leading to fundamental damage through forming atheromatous or fibro-fatty plaque [24]. Atherosclerosis is a result of respond of innate immunity involving macrophages, which is initiated by arterial endothelium cells that respond to modified lipoproteins leading to Th1 cells subset activation and generation of inflammatory cytokines and chemokines [25], and such result is in agreement with the results of Eichbaum and colleagues [26], who demonstrated that warfarin exerts an anti-inflammatory action in experimental rodents and this effect was not related to the anticoagulant properties of warfarin. In this regard, these reports supported by other studies which explain that warfarin exert its action on vitamin K metabolism by blocks one of the enzymes that uses vitamin K to make some of clotting factors [27], in addition to its action on cellular physiology independent to its action on vitamin K. Moreover, another studies indicate that the inhibition of 1-KB phosphorylation at lower concentrations of warfarin and then stimulation of it at higher concentrations suggest that warfarin could directly influence inflammatory gene transcription in 4/4 macrophages in murine clone [28], this studies supported our results because macrophages are the main source of cytokines in the atherosclerotic plaque, including pro-inflammatory cytokines TNF-α, IL-1, IL-6, IL-12,IL-15, and IL-18 as well as the anti-inflammatory cytokines IL-10 and IL-32. Increasingly, more studies have shown that low concentrations of warfarin inhibit interleukin-6 production in mice [29]. In addition, warfarin exerts influence on some activities, including anti-tumor and immunomodulating effects, hence warfarin can
influence on both immune stimulating and suppressive activities have been observed in different experimental systems [30].

Conclusion

According to the present study we conclude that high dose warfarin intake as oral anticoagulant drug in treatment and prevention of thrombi in deep venous thrombosis disease may be considered one of the reasons which influence inflammatory process by stimulating two of the most important pro-inflammatory cytokines involving IL-6 and TNF-α in atherosclerosis disease.

Recommendation

We were recommended to more investigation about new anticoagulant drugs for treatment of deep venous thrombosis disease which have less effect on atherosclerosis, these types of studies needed more cooperation between the authors, special physicians and medical laboratories because such studies very useful to the patients and physicians to evaluate the exact role of the widely used drug warfarin in treated of deep venous thrombosis disease in addition to control side effects of this drug.

References


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