Evaluation of Cardiovascular Risk Factors in Tunisian Coronary Patients

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Abstract

Introduction: Ischemic heart disease ranked among the leading causes of death worldwide. Several biological and genetic risk factors associated with acute coronary syndrome. By addressing the risk factors, it is possible to prevent most of the cardiovascular diseases and contribute to the reduction of complications of acute coronary syndrome. Material and Methods: Our study is a prospective study that was conducted to the main military hospital of instruction of Tunis. Recruitment involved 122 coronary (n = 122) and 705 controls (n = 705) totaling a population of 827 subjects. Data collection concerned socio-demographic characteristics, anthropometric measurements, habits and lifestyle, health status and biological assessment. Results: Our study reported a high prevalence of cardiovascular classic risk factors particularly overweight (62.3%), diabetes (63.1%) and hypertension (50%). In 55% of coronary patients, moderate to intermediate hyperhomocysteinemia was found, but it appears to be an independent risk factor. The results of our study show a significant difference in the genotypic frequencies of the C677T mutation in the MTHFR gene between the two populations, cases and controls. Conclusion: Acute coronary syndromes are the leading cause of sudden death in adults. The evaluation of risk factors after acute coronary syndrome episode is essential for a better management according to the recommendations of learned societies and standards of good practice.

Subject Areas
Cardiology

Keywords
Acute Coronary Syndrome, Cardiovascular Risk Factors, Homocysteine,
1. Introduction

According to High Health Authority, estimates of the incidence of acute coronary syndromes (ACS), report 2500 SCA per million inhabitants [1]. In 2012, the World Health Organization (WHO) [2] classified ischemic heart disease as one of the top 10 causes of death in the world with a rate of 7.4 million deaths. This rate increases exponentially according to the age of the patients and according to WHO [3], it affects almost equally men and women.

The impact in terms of public health and health expenditure is major, indeed in 2008, 214,000 people were hospitalized at least once for ischemic heart disease [4]. By addressing risk factors such as smoking, unhealthy diet and obesity, high blood pressure, diabetes and hyperlipidemia, it is possible to prevent most cardiovascular disease (CVD) and contribute to decrease the complications of SCA.

In this context, it seemed appropriate to study classical risk factors and to evaluate new risk factors in a group of Tunisian coronary patients. We have also studied the association between these risk factors and their implications for the development of CVD.

2. Material and Methods

2.1. Study Design and Subjects

This study was conducted on a population of 122 CAD patients, admitted to the Cardiology service of the Military Hospital of Instruction of Tunis from January to December 2016. Also, a group of 705 healthy individuals with neither symptoms nor previous diagnosis of cardiovascular problem was studied as control group. They were recruited from medical and paramedical volunteers. Statistical population number was determined by power calculation. We didn’t find the exact number but we couldn’t collect more during the mentioned period.

Treatment for inflammatory or chronic infectious disease or malignancy was an exclusion criterion. Individuals with CRP values above 10 mg/ml were excluded from the analyses, due to the possibility of an acute infection. Clinical information, including age, sex, BMI, smoking, and alcohol consumption, were obtained from patients’ medical charts.

2.2. Blood Sampling

Venous blood sample collected from each subject after informed consent for biochemical and molecular assays.

2.3. Biochemical Assays

Serum triglycerides, serum cholesterol and high-density lipoprotein (HDL)-cholesterol were assayed by an enzymatic-colorimetric technique using
Unicell DXC 800 analyzer (Beckmann Coulter). Low-density lipoprotein (LDL)-cholesterol was calculated using the Friedwald’s formula.

Serum concentration of hs-CRP, ApoA1, ApoB and Lp(a) were assayed by an immuno-nephelometric method using the BN II nephelometer Analyzer.

The total homocysteine, B12 vitamin and folate assays were carried out according to the immuno-chemiluminescent technique performed by the Immulite® analyzer (Siemens, Germany) based on a competitive immunoassay.

2.4. DNA Extraction

Genomic DNA was extracted from intravenous blood, using the salting out method of DNA. This method involves salting out of the cellular proteins by precipitation with a saturated NaCl solution.

2.5. Genotype Determination

The MTHFR C677T gene polymorphism was genotyped by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism method (PCR-RFLP). The PCR reaction was performed in a final volume of 50 µl containing equal concentration (10 picomoles) of Forward Primer 5’-TGAAGGAGAAGTTGCTGCGGGA-3’ and reverse primers 5’-AGGACGTTGCGGTAGAGTG-3’ (Designed using Primer 3 software), 25 mM desoxynucleotide triphosphate, PCR Buffer, 1.25 U of Taq polymerase (Agilent).

The amplification is carried out in 40 cycles of 1 min of denaturation at 94°C, 1 min of annealing and 1 min of extension at 72°C. A last cycle of 72°C for 10 min fixes the end of the elongation and thus completes the PCR reaction.

2.6. Digestion of the PCR Product

PCR products are subjected to enzymatic digestion by the restriction enzyme Hinfl (Thermo Scientific). It recognizes and cleaves the sequence 5’-G A N T C-3’, while the mutated sequence is not recognized by the enzyme nor cut at this level. The stage of the enzymatic digestion is carried out in a final volume of 30 µL which contains 10 µL of PCR product, a buffer, bidistilled water, and the restriction enzyme Hinfl. The mixture is incubated for 5 min at 37°C and the inactivation of the enzyme is at 65°C for 20 minutes. The obtained fragments’ sizes were analyzed on a 1.2% agarose gel for 90 min.

2.7. Statistical Analysis

The statistical analysis of various data collected was carried out using the SPSS software version 20.0 (SPSS Inc, USA) for Windows (Microsoft Corporation, USA). Quantitative variables were compared by the Student’s t-test and the qualitative data by the Chi-square test. The Spearman test was used to study the correlation between the different parameters studied. The threshold of significance was set at 0.05.
2.8. Ethical Consideration

Participation was voluntary and verbal consent was acquired from each participant prior to sample collection. Confidentiality of all participants was maintained as no names were requested. The study was approved by the local ethics committee.

3. Results

Our study included 827 subjects divided into 2 groups: A group of 705 control subjects and a group of 122 coronary patients. Anthropometric parameters for patients and controls are shown in Table 1.

The mean age of our sample was 63 ± 10.06 years. The difference with the control group (47.99 ± 9.021 years) is statistically significant (p < 0.05).

Disease distribution by sex showed that men are more affected than women.

In coronary patients, overweight or obese subjects are 76 with a mean BMI = 26.6 ± 3.84 Kg/m² and with a statistically significant difference compared to the control group.

It was noted that 27.9% of patients had a family history of cardiovascular disease. Smoking was less common in controls compared to patients (54.3% vs 58.2%), but without statistically significant difference (p = 0.428). Of the CAD group (n = 122), a large proportion of patients have diabetes (n = 61) and 77 are hypertensive.

The comparison of lipid parameters between controls and coronary patients is presented in Table 2.

The levels of cholesterol between the two groups were compared. In fact biochemical analyzes showed that TC levels were higher in the coronary group (4.58 ± 1.30 mmol/L) than controls (3.98 ± 1.05 mmol/L) with a statistically significant difference.

Table 1. Anthropometric parameters of the two studied groups.

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 705)</th>
<th>Patients (n = 122)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>47.99 ± 9.02</td>
<td>63.86 ± 10.06</td>
<td>&lt;10⁻³</td>
</tr>
<tr>
<td>Men</td>
<td>83.8%</td>
<td>63.1%</td>
<td>&lt;10⁻³</td>
</tr>
<tr>
<td>Women</td>
<td>16.2%</td>
<td>36.9%</td>
<td></td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>24.42 ± 3.35</td>
<td>26.61 ± 3.84</td>
<td>&lt;10⁻³</td>
</tr>
<tr>
<td>BMI &gt; 25</td>
<td>43.8%</td>
<td>62.3%</td>
<td>&lt;10⁻³</td>
</tr>
<tr>
<td>Family history</td>
<td>21.1%</td>
<td>27.9%</td>
<td>0.005</td>
</tr>
<tr>
<td>Tobacco</td>
<td>54.3%</td>
<td>58.2%</td>
<td>0.428</td>
</tr>
<tr>
<td>Alcohol</td>
<td>12.9%</td>
<td>2.5%</td>
<td>0.001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0%</td>
<td>50%</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>0%</td>
<td>63.1%</td>
<td></td>
</tr>
</tbody>
</table>

BMI: Body Mass Index; p: significance.
between the 2 groups with a higher mean in the patients groups. In our sample, there is an overall decrease in HDL-c concentrations with an average of 0.94 ± 0.32 mmol/L.

**Figure 1** shows that hyperhomocysteinemia was more frequently present in coronary artery disease patients than controls.

Plasma homocysteine levels varies between 9.35 and 25.99 μmol/L. Hyperhomocysteinemia was present in our study population in 67 patients with a statistically significant difference compared to controls ($p < 0.005$).

Of 122 coronary patients, 72 had a CRP elevation, a high hs CRP values are significantly more elevated in coronary patients compared to controls (Table 3).

Statistical analysis has shown that increased coronary homocysteine levels correlate with increased CRP. The correlation line is shown in **Figure 2**. ($r = 0.23, p = 0.01$).

**Table 4** shows the comparison of genotype frequencies between controls and patients.

We found that the prevalence of the MTHFR C677T mutation is significantly higher in patients, especially for the CT genotype.

**Table 2.** Comparison of lipid parameters between controls and coronary patients.

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 705)</th>
<th>Patients (n = 122)</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mmol/L)</td>
<td>3.98 ± 1.05</td>
<td>4.58 ± 1.03</td>
<td>$&lt;10^{-3}$</td>
</tr>
<tr>
<td>LDLc (mmol/L)</td>
<td>2.34 ± 1.18</td>
<td>2.96 ± 1.01</td>
<td>$&lt;10^{-3}$</td>
</tr>
<tr>
<td>HDLc (mmol/L)</td>
<td>0.97 ± 0.28</td>
<td>0.94 ± 0.32</td>
<td>0.215</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.10 ± 0.5</td>
<td>1.45 ± 0.80</td>
<td>0.103</td>
</tr>
<tr>
<td>Lp(a) (g/L)</td>
<td>0.11 ± 0.11</td>
<td>0.36 ± 0.25</td>
<td>$&lt;10^{-3}$</td>
</tr>
<tr>
<td>Apo A1 (g/L)</td>
<td>1.19 ± 0.27</td>
<td>1.23 ± 0.25</td>
<td>0.111</td>
</tr>
<tr>
<td>Apo B (g/L)</td>
<td>0.74 ± 0.26</td>
<td>0.99 ± 0.31</td>
<td>$&lt;10^{-3}$</td>
</tr>
<tr>
<td>Apo B/Apo A1</td>
<td>0.69 ± 1.67</td>
<td>0.82 ± 0.26</td>
<td>0.419</td>
</tr>
</tbody>
</table>

TC: Cholesterol; LDLc: Low Density Lipoprotein cholesterol; HDLc: High Density lipoprotein cholesterol; TG: Triglyceride; Lp(a): Lipoprotein(a); Apo A1: Apolipoprotein A1; Apo B: Apolipoprotein B; $p$: significance.
Table 3. Comparison of hs-CRP levels between the two studied groups.

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Patients</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hs-CRP (mg/L)</td>
<td>2.11 ± 3.15</td>
<td>14.64 ± 10.04</td>
<td>&lt;10(^{-3})</td>
</tr>
</tbody>
</table>

Hs-CRP: high sensitivity C reactive protein; \( p \): significance.

Table 4. Genotype distribution of MTHFR C677T polymorphism in coronary artery disease patients and control subjects.

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Patients</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>50.0%</td>
<td>28.1%</td>
<td>&lt;10(^{-3})</td>
</tr>
<tr>
<td>CT</td>
<td>42.7%</td>
<td>56.3%</td>
<td>0.013</td>
</tr>
<tr>
<td>TT</td>
<td>7.3%</td>
<td>15.6%</td>
<td>&lt;10(^{-3})</td>
</tr>
<tr>
<td>C allele</td>
<td>71.35%</td>
<td>56.25%</td>
<td>&lt;10(^{-3})</td>
</tr>
<tr>
<td>T allele</td>
<td>27.65%</td>
<td>43.75%</td>
<td>&lt;10(^{-3})</td>
</tr>
</tbody>
</table>

To study the effect of mutated genotypes on homocysteinemia, its plasma concentrations were compared between the CC, CT and TT genotypes in all of our subjects. The results obtained (in Figure 3) reveal that there was no significant difference in Hcy levels between the CC, CT and TT genotypes. However, the highest Hcy values are observed in TT genotype patients.

Table 5 reveals that the concentrations of Folates and B12 vitamin were comparable in the different groups and independent of the genotype of MTHFR.
Figure 3. Interaction of C677T MTHFR Polymorphism of with Homocysteine.

Table 5. Association of CC, CT and TT genotypes with homocysteine, folate and B12 vitamin.

<table>
<thead>
<tr>
<th></th>
<th>CC</th>
<th>CT</th>
<th>TT</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hcy (μmol/L)</td>
<td>18.83 ± 4.78</td>
<td>18.12 ± 5.21</td>
<td>21.22 ± 9.63</td>
<td>NS</td>
</tr>
<tr>
<td>Folate (ng/mL)</td>
<td>7.78 ± 3.25</td>
<td>7.15 ± 4.36</td>
<td>7.80 ± 6.84</td>
<td>NS</td>
</tr>
<tr>
<td>Vitamin B12 (pg/mL)</td>
<td>393 ± 247.22</td>
<td>343.22 ± 220.26</td>
<td>450 ± 326.91</td>
<td>NS</td>
</tr>
</tbody>
</table>

4. Discussion

Several studies have shown that the incidence of cardiovascular disease is increasingly important due to the accumulation of several risk factors. [5].

In our study, cardiovascular risk increased with age. It has been proven by the Framingham study and the MONICA project that the risk of coronary heart disease increases markedly with age [6] [7].

Coronary artery disease does not spare women, although in its usual presentation, they benefit from natural hormonal protection that explains why they enter coronary disease 10 years later than men [8]. Our study shows an increase in disease incidence with age for both sexes. Male sex represents 63.1% of coronary heart disease patients. This has been confirmed by M. Seck et al. [9] in sub-Saharan Africa in 2007.

Genetic factors also play an important role in determining the risk of coronary heart disease. It appears that subjects with a family history of coronary artery disease are more exposed than the rest of the general population [10]. Only early cardiovascular accidents in the father, mother or first-degree relative should be considered (before age 55 in men and before age 65 in women). In our study 27.9% of our patients had a family history of cardiovascular disease compared to only 21.1% in the control group, this difference is statistically significant. The Framingham study found that the occurrence of a coronary death in a parent increases the risk of coronary heart disease in children by 30% [6].

Active chronic smoking has emerged as a predominant risk factor. In our
study 58.2% of coronary patients are smokers, with a higher prevalence in the male sex. Our results are consistent with those found in the Tunisian Sahel in 2007 [11] and in Greater Tunis between 2004 and 2005 [12]. In the INTERHEART study, the share of smoking for the risk of myocardial infarction is 36.5% and it occupies a second position after dyslipidemias [13]. The toxicity of cigarette smoke on the arterial wall involves three main mechanisms: atherogenesis, thrombogenesis and spasm.

Obesity is associated with an increased risk of coronary heart disease. In our study 62.3% of patients were obese or overweight. Our results are higher compared to Ariane Sultan’s study (16%) [14]. In a recent meta-analysis, there was a 29% increase in the risk of coronary disease for every five-point increase in BMI [15].

The high prevalence of diabetes (63.1%) in our population is significantly higher than that reported in Japan in 2013 (13.9%) [16] and that found in a Tunisian study (12.5%) [17]. In our population, diabetes has emerged as the most frequent risk factor. In coronaryography, Merzouk et al. [18] found that trituncular involvement was more common in diabetics, while monotunricular involvement was more common in non-diabetics.

In this study, 50% of our sample was hypertensive, with females being widely represented. The female predominance of hypertension could be explained by the multifactorial association in women including obesity, sedentary lifestyle, contraceptive use… The high prevalence of estimated hypertension in this population is higher than that reported in previous Tunisian studies (29%) [19] and in other developing and developed countries (29.6%) [20]. The harmful role of diastolic blood pressure (DBP) was first highlighted, but it is now known that systolic blood pressure (SBP) has the strongest prognostic significance. More recently, the particularly deleterious role of pulsed pressure (or differential pressure = PAS – PAD) has been highlighted. Its increase reflects an alteration of the compliance (or damping function) of large vessels.

In the literature, numerous cohort studies and Meta analyzes have established the relationship between total cholesterol, LDL-c, HDL-c and triglycerides with cardiovascular risk.

In our study, hypercholesterolemia was observed in 27.9% of coronary patients. Its prevalence increases with age, however our results remain lower than those found in industrialized countries (56%) [21].

Excess LDL-c plays a key role in the onset and development of atherosclerosis. Indeed, LDL-c accumulates, is fixed in the arterial wall and oxidizes, which contributes to an inadequate immune response to the origin of an inflammatory phenomenon related to the secretion of chemokines, the transformation of macrophages into “Scavengers” and the arrival of T cells that release pro-inflammatory cytokines. Inflammation becomes permanent and creates an atherosclerotic plaque. Thin and inflamed plaques containing a lot of lipids and macrophages are the most likely to break. In our study the increase in LDL-c af-
fected 32% of all coronary patients, our results are consistent with those found in a study conducted in Mauritius in 2012 [22].

In our study 32% of our patients had a decrease in HDL-c. It has protective effects by ensuring the return of cholesterol and its hepatic cleansing. They also have the property of limiting the oxidation of LDL-c and thus allow to a certain extent to fight against the formation of foam cells [23].

An increase in Lp(a), a macromolecule consisting of an LDL and an Apo(a) glycoprotein, was found in 58.2% of cases with an average of 0.396 g/L. A recent study has shown that Lp(a) concentrations above 300 mg/L have been associated with a gradual increase in risk [24].

In our study, the frequency of hyperhomocysteinemia is more important in coronary patients. Our results are consistent with a study in a Tunisian population that showed that subjects with Hcy > 13 μmol/L are about twice as likely to have significant coronary stenosis as subjects with Hcy < 9 μmol/L [25].

Homocysteine may promote LDL-c oxidation, vascular smooth muscle cell proliferation, platelet and coagulation factors activation, and endothelial dysfunction. As a result, abnormalities in homocysteine metabolism are now receiving increasing attention because of their potential role in the pathogenesis of atherosclerosis and other diseases such as venous thrombosis [26].

In the present investigation, a positive correlation was found between markers of inflammation and severity of acute coronary syndrome. CRP has a predictive power of complications in patients with acute myocardial infarction, stable or unstable angina [27]. Epidemiological studies have demonstrated the role of elevated CRP as an independent risk factor for myocardial infarction in men with or without other risk factors [28]. This suggests the pathogenic role of inflammation in the genesis of acute coronary syndrome. On the other hand, we also found that the increase in CRP was positively correlated with hyperhomocysteinemia. Hcy stimulates the production of several pro-inflammatory molecules, this has been documented by various in vitro studies. Lesions induced by homocysteine via increased production of adhesion molecules, cytokines, and chemokines may contribute to the maintenance of chronic inflammation, a key factor associated with the development of atherosclerosis [29].

Genetically, and according to our results, CT and TT genotypes are significantly more common in coronary patients compared to controls. Our results are higher than those reported in an Algerian study; indeed in this study the authors reported a TT genotype frequency of 6% in a coronary group. However, our results are close to those found by Jerbi et al. [30] (17.8%).

5. Conclusion

In addition to conventional risk factors, new risk factors such as inflammation, hyper homocysteinemia and certain genetic polymorphisms deserve to be considered in the monitoring of cardiovascular diseases. The use of these new markers in the evaluation of cardiovascular risk, especially in young adults in the
absence of classical factors, seems to be essential and could partly explain the deficiencies of conventional means of assessing cardiovascular risk. Homocysteine therefore deserves to be measured in the presence of vascular pathologies, regardless of the age, sex and conventional risk factors that the patient presents.

References


Tun Med, 89, 621-626.

