Correlation of Ischemia Modified Albumin and Advanced Oxidation Protein Products with lipid profile: A Possible link to cardiovascular diseases

Arun Kumar K.a, Sheila Uthappa b, Sudarshan Surendran c, Avinash S.S. d, Sucharitha Suresh e, Sushitha E.S. d

a Department of Biochemistry, Fr. Muller Medical College, Mangalore – 575002, Karnataka, India
b Department of Biochemistry and Biophysics, St. John’s Medical College, Bangalore – 560034, Karnataka, India
c Department of Anatomy, Melaka Manipal Medical College, Manipal University, Manipal – 576104, Karnataka, India
d Department of Biochemistry, Fr. Muller Medical College, Mangalore – 575002, Karnataka, India
e Department of Hospital Administration, Fr. Muller Medical College, Mangalore – 575002, Karnataka, India

Abstract

Aim: To evaluate the presence of a correlation between Ischemia Modified Albumin (IMA) and Advanced Oxidation Protein Products (AOPP) to cardiovascular diseases. Study design: Our study was designed to evaluate any link between the levels of IMA and AOPP with other risk factors known to increase risk of cardiac diseases (such as lipid profile). Place and duration of the study: This study was conducted by including the patients visiting Father Muller Medical College Hospital, Mangalore, Karnataka, India. The study was conducted for one year from May 2012 to April 2013. Methodology: A total of 151 participants were categorized into normal, prehypertension and hypertension groups. They were assessed for their levels of AOPP and IMA, and then these values were correlated to various other basic parameters. Results: On a whole, when the results of all the three groups were combined and AOPP correlated with the BMI, lipid profile values and IMA, a significant positive correlation (P<0.05) was seen with BMI, LDL (bad cholesterol), Triglycerides, TCHOL/HDL ratio, and NONHDL. Conclusion: The increase in IMA and AOPP levels indicate a positive correlation with most of the lipid profile values, which are known risk factors in cardiovascular diseases.


All Rights Reserved with Photon.
Photon Ignitor: ISJN44385728D753203042015

1. Introduction

There are well-known and reported risk factors that have been disposed as the causative factors for cardiovascular diseases or myocardial infarction. Some of them include hyperlipidemia or...
dyslipidemia, hypertension, diabetes mellitus, obesity and also lifestyle (mostly sedentary) (Gordon et al., 1977; Kannel et al., 1976). Considering the markers for oxidative stress, the products of lipid peroxidation among the indices of oxidative damage are the most preferred markers. There are various biomarkers used for the detection of ACS development at its different stages (Apple et al., 2005; Tsakiris et al., 2006). Based on the usage of these biomarkers, they can be categorized into either the risk stratification type or the diagnostic type (Sbarouni et al., 2008). Lipid profile has been linked to various heart conditions in the literature and it is seen in various experiments and the hypothesis of lipid relation to cardiac diseases is emphasized by the presence of high levels of cholesterol in such diseased conditions both in animals and humans (Armstrong et al., 1970; Connor, 1961; Jagannathan et al., 1974; Manson et al., 1992).

It is seen in the literature that dyslipidemia has been stated as one of the important risk factors in cardiovascular diseases and also linked to the advancement to atherosclerosis, which might lead to other diseases associated with cardiovascular diseases (Berenson et al., 1989; Zieske et al., 2002). Myocardial infarctions, coronary artery disease, stroke, as a result of atherosclerosis have increased in considerable proportions in the middle age and elderly group of people. This has been seen to be increasing considerably in the industrialized countries and accounts to around 50% of all death occurring in these regions (Allender, 2008; American Heart Association, 2002). Cardiovascular diseases (CVDs) are presently the leading causes of death in industrialized countries and expected to become so in emerging countries by 2020. (Murray et al., 1997) In fact, CVDs would be the single largest cause of death in the world accounting for more than a third of all deaths. (Thygesen et al., 2007) In India, Cardiovascular diseases are the largest cause of mortality, accounting for around half of all deaths resulting from non-communicable diseases. Overall, CVDs accounted for around one-fourth of all deaths in India in 2008. In addition, Cardiovascular disease is responsible for loss of 10% of healthy years of life in low- and middle-income countries, and 18% in high income countries.

The importance of proteins playing a vital role in the living organisms is well reported. Their stringent dependency on particular folding pattern and structure makes their functioning of particular interest. Such proteins with modifications could be resourcefully used when compared to other biomarkers in monitoring advancement of diseases and their effects. As the functional capabilities of proteins depends on the precise folding and innate structure, any modification in their conformation or structure in any unfavorable conditions (such as oxidative stress, inflammation etc.) could lead to their functional inability, loss, or even inhibition of degradation (leading to accumulation). When seen down the functional hierarchy, these hindrances in their normal functioning can lead to changes in the internal environment of the cells, in turn leading to damaged tissues and finally on a whole to the onset of a disease and might be to the progress of the same too. The estimation of such modified proteins, especially AOPP being simple and cost effective, can be of considerable importance if the relation between BMI, lipid profile and IMA could be elucidated.

Advanced Oxidation Protein Products (AOPPs) have been extensively researched and they show a promising marker for imbalance in oxidative stress condition, owing to their stability and the ease of sampling (Mera et al., 2005). Transformation of macromolecules such as proteins results in the formation of compounds such as Advanced Glycation End products (AGEs) and Advanced Oxidation Protein Products (AOPPs). The derivation of AOPPs is from albumin modified due to oxidation and also lipoproteins. One of the main elements involved in such modifications is believed to be oxidative stress and is correlated to various diseases along with the related complications (Kalousova et al., 2005; Piwowar, 2010).

It has been reported that there are a number of factors that influence the levels of IMA in serum and in particular these levels are related to serum albumin and serum lipid levels (Roy et al., 2004). Some of the other diseases that are relatively related to increased levels of serum lipids do also present with increases in the levels of IMA. There are various factors that could cause ischemia, such as sodium pump disruption, calcium pump disruption, free radical injury, acidosis and hypoxia to mention some. In such cases, the amino terminal of albumin might be modified for the binding of metals such as copper, nickel and cobalt that result in the formation of modified albumin.

The above said is in relation to the use of BMI, lipid profile as markers for various conditions. Keeping in mind the above said, this study was designed as an initial step to finding out the relation (if any) between AOPP and IMA, with the above said parameters. These correlations were done on three groups of participants (normal, prehypertension and hypertension). The analyses of correlations between the various parameters have been discussed.
2. Objectives of Research

1. To study the correlation of IMA and the established risk factors of cardiovascular diseases in pre-hypertensive and hypertensive patients.
2. To study the correlation of AOPP and the established risk factors of cardiovascular diseases in pre-hypertensive and hypertensive patients.
3. Correlation of ischemia marker IMA and product of protein oxidation AOPP in normal, prehypertensive and hypertensive patients.

Hypertension, obesity, decreased HDL and elevated Triglycerides significantly increase the risk for cardiovascular diseases and atherosclerosis. While oxidative stress is well established in conditions like obesity, hypertension and dyslipidemia, only a few data show oxidative modification of albumin in these conditions (Sebekova et al., 2006). Albumin undergoes oxidation with different agents in different ways and at different sites. Oxidative stress induced structural modifications of albumin not only alters its binding properties but also alters its functions. Modified forms of albumin are believed to be involved in the pathogenesis of disorders involving oxidative stress like diabetes mellitus. As both AOPP and IMA are formed by the oxidative modification of albumin, their relationship with cardiovascular risk factors gives information on whether or not they have any role to play in the pathogenesis of cardiovascular diseases.

3. Materials and Methods

This hospital based study was done at Father Muller Medical College Hospital Mangalore, which is located in Karnataka state of India. The study duration was from December 2011 to June 2013. Patients visiting the outpatient department for routine health check up were the subjects of this study. Patients were divided into normal, prehypertension and hypertension groups based on blood pressure. The age group of the patients was 23 to 73 years, and both males and females were included in the study. Lipids, AOPP, IMA are estimated in the fasting blood samples collected from these patients and BMI is calculated using height and weight.

3.1 Patient data collection

This study was conducted by including the patients visiting Father Muller Medical College Hospital; Mangalore, Karnataka, India. Based on the number of patients visiting the hospital and fitting in for the study, the study was designed and the protocol stated. The patients were informed about the study and their consent was taken before they were then included in the study. The study design and protocol has been approved by the institutional ethical committee.

3.2 Sample size and categorization

A total of 151 (n) patients were included in the study. Based on their blood pressure levels, the samples were grouped into normal, prehypertension and hypertension groups. Individuals with systolic blood pressure less than 120 mm Hg and diastolic pressure less than 80 mm Hg were grouped as normal. Prehypertension group included patients with systolic pressure between 120 and 139 mm Hg and diastolic pressure between 80 and 89 mm Hg. The third group namely hypertension patients had systolic pressure of 140 mm Hg or more and diastolic pressure of 90 mm Hg or more.

3.3 Exclusion criteria

Patients with increased serum creatinine (>1.5mg/dl) and those with decreased serum albumin levels (<3g/dl) were excluded from the study.

3.4 Sample collection

An informed consent was obtained from healthy individuals and their fasting blood samples were collected in red-top vacuum tubes. Along with the blood, the blood pressure and clinical history was also collected. The instrument, Cobas c311 was used for the immediate estimation of serum lipid profile, glucose, urea, creatinine and albumin levels. Calculation of total cholesterol/HDL ratio and non-HDL were done based on the lipid profile values obtained from the above estimations. Until the estimation of AOPP and IMA, the samples were stored at -20°C.

3.5 Estimation of AOPP

Determination of AOPP was based on spectrophotometric detection according to Witko-Sarsat et al. (Witko-Sarsat et al., 1996). Diluted blood serum - 1000 µl with PBS (1:5 ratio), 1000 µl of chloramin T (0-100 µmol/l) for calibration and 1000µl of PBS as blank were pipetted into separate test tubes. 50 µl of 1.16 M KI and 100 µl of acetic acid were added and absorbance at 340 nm was measured immediately. Concentration of AOPP is expressed in chloramines T units (µmol/l).
4. Results

4.1 Results for correlation of AOPP with lipid profile and other basic parameters

<table>
<thead>
<tr>
<th>Parameter I</th>
<th>Parameter II</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>AOPP</td>
<td>BMI</td>
<td>0.179</td>
<td>0.333*</td>
<td>0.134</td>
<td>0.151*</td>
</tr>
<tr>
<td></td>
<td>TCHOL</td>
<td>0.353**</td>
<td>-0.118</td>
<td>-0.07</td>
<td>0.062</td>
</tr>
<tr>
<td></td>
<td>HDL</td>
<td>-0.353**</td>
<td>-0.373*</td>
<td>-0.373**</td>
<td>-0.331***</td>
</tr>
<tr>
<td></td>
<td>LDL</td>
<td>0.367**</td>
<td>0.267***</td>
<td>0.255</td>
<td>0.247**</td>
</tr>
<tr>
<td></td>
<td>TG</td>
<td>0.695***</td>
<td>0.689*</td>
<td>0.554***</td>
<td>0.647***</td>
</tr>
<tr>
<td></td>
<td>TCHOL/HDL</td>
<td>0.606***</td>
<td>0.119</td>
<td>0.272*</td>
<td>0.332***</td>
</tr>
<tr>
<td></td>
<td>NONHDL</td>
<td>0.481****</td>
<td>-0.281*</td>
<td>0.001</td>
<td>0.148*</td>
</tr>
<tr>
<td></td>
<td>IMA</td>
<td>-0.379**</td>
<td>-0.114</td>
<td>-0.154</td>
<td>-0.219**</td>
</tr>
</tbody>
</table>

Group I – Normal Control, Group II – Prehypertension, Group III – Hypertension, Group IV - Normal + Prehypertension + Hypertension Group. [* - P value < 0.05, ** - P value < 0.01, *** - P value < 0.001]

4.2 Results for correlation of IMA with lipid profile and other basic parameters

<table>
<thead>
<tr>
<th>Parameter I</th>
<th>Parameter II</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMA</td>
<td>BMI</td>
<td>-0.291*</td>
<td>-0.036</td>
<td>0.397**</td>
<td>0.025</td>
</tr>
<tr>
<td></td>
<td>TCHOL</td>
<td>-0.04</td>
<td>0.018</td>
<td>0.293*</td>
<td>0.083</td>
</tr>
<tr>
<td></td>
<td>HDL</td>
<td>0.171</td>
<td>0.012</td>
<td>0.393**</td>
<td>0.162*</td>
</tr>
<tr>
<td></td>
<td>LDL</td>
<td>-0.061</td>
<td>-0.005</td>
<td>0.121</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td>TG</td>
<td>-0.445**</td>
<td>-0.155</td>
<td>-0.039</td>
<td>-0.228**</td>
</tr>
<tr>
<td></td>
<td>TCHOL/HDL</td>
<td>-0.152</td>
<td>-0.001</td>
<td>-0.027</td>
<td>-0.057</td>
</tr>
<tr>
<td></td>
<td>NONHDL</td>
<td>-0.092</td>
<td>0.014</td>
<td>0.221</td>
<td>0.044</td>
</tr>
<tr>
<td></td>
<td>AOPP</td>
<td>-0.379**</td>
<td>-0.114</td>
<td>-0.154</td>
<td>-0.219**</td>
</tr>
</tbody>
</table>

Group I – Normal Control, Group II – Prehypertension, Group III – Hypertension, Group IV - Normal + Prehypertension + Hypertension Group. [* - P value < 0.05, ** - P value < 0.01]

5. Discussion

In our study, an attempt has been made to relate the levels of AOPP and IMA to the basic lipid profile values and there exists a positive correlation of both AOPP and IMA values with most of the lipid profile values. There have been many reports in the literature stating the relation of levels of AOPP (Anderstam et al., 2008; Bayati et al., 2013; Chiu-Braga et al., 2006; Piwowar et al., 2007) and IMA (Roy et al., 2004; Sinha et al., 2003; Van Belle et al., 2010) to various major conditions. If the pathophysiology of AOPP and IMA could be better understood, it could provide a broader picture of their involvement in different disease conditions. A major link has been established between the levels of AOPP and other markers for cardiovascular diseases. The lipid profiles are also shown to play a vital role in cardiovascular diseases and this relation has been extended to the level of using them as biomarkers (Bhatia et al., 2013; Bogavac-Stanojevic et al., 2007; Law et al., 2011). If this correlation is further extended, it could provide a valuable source of information in the prognosis or diagnosis of cardiovascular diseases.

The participants in our study were grouped into normal, pre-hypertensive and hypertensive groups based on their blood pressure levels, the above said parameters were analyzed and the values were correlated.

5.1 Comparison of AOPP and IMA levels in all the groups

On a whole when the results of all the three groups (normal, prehypertension and hypertension) [Table 01], were combined and AOPP correlated with the BMI, lipid profile values and IMA, it was seen that, AOPP had a positive correlation resulting in their elevated levels in most of these values, except for HDL and IMA which remained the same. This positive correlation was significant (P < 0.05) with BMI, LDL, TG, TCHOL: HDL ratio, and NONHDL. However, there was a significant (P<0.05) negative correlation with HDL and IMA that would result in the reduction of HDL and IMA with rise in the AOPP levels. On the other hand, IMA [Table 02] was seen to have a significant (P <0.05) positive correlation with HDL and a significant (P<0.01) negative correlation with TG. The other parameters in case of IMA showed changes but there was no significance observed.

5.2 Comparison of AOPP and IMA levels in the normal group

In the normal group [Table 1] showed a significant (P<0.05) positive correlation to BMI, LDL, TG,
TCHOL: HDL ratio and NONHDL. However, the correlation was negative with respect to HDL and IMA. With respect to IMA [Table 02], except HDL showing a positive correlation, all other parameters were seen to have a negative correlation with IMA.

5.3 Comparison of AOPP and IMA levels in the prehypertension group
In the prehypertension group [Table 01], there was a significant ($P<0.05$) positive correlation between BMI, LDL and TG. In case of the TCHOL: HDL ratio, there was a positive correlation but no significance was seen in the correlation. On the contrary in this group, the TCHOL, HDL, NONHDL and IMA showed a negative correlation with AOPP values. In this group, IMA [Table 02] showed a negative correlation to most of the parameters except for TCHOL, HDL and NONHDL, where it showed a positive correlation. However, none of the correlations of IMA were significant in the prehypertension group.

5.4 Comparison of AOPP and IMA levels in the hypertension group
In the hypertension group [Table 01], BMI and LDL showed a positive correlation but there was no significance seen. With TG, TCHOL: HDL ratio and NONHDL, AOPP showed a significant ($P < 0.05$) positive correlation. IMA [Table 02] was seen to have a positive correlation with many of the parameters in this group when compared with the other groups. The triglycerides, TCHOL: HDL ratio and AOPP were seen to have a negative correlation. There was a significant ($P < 0.05$) positive correlation between IMA and BMI, TCHOL and HDL. The correlation between LDL, NONHDL was positive with IMA but did not show any significance.

5.5 Overall comparison
HDL as a whole was seen to be unaffected in all the groups i.e., in the normal, prehypertension, hypertension and the combined group. The result suggests that there is a direct relation between the values of AOPP and LDL and a significant positive correlation between the two raises an alarming question of controlling the AOPP levels in order to maintain the levels of LDL (the bad cholesterol) in control. More the increase in AOPP levels, there could be an increase in the levels of LDL. This increase in the levels of LDL has been proved to be risky with respect to cardiovascular diseases (Andreoli et al., 1982; Rudling, 2006). These results suggest that there is a direct influence of the formation of AOPPs on the BMI, lipid profile except HDL and IMA. A direct influence of AOPP on the BMI, LDL, TG, TCHOL: HDL ratio and IMA could possibly be extended with more understanding of the relation between AOPP and other parameters that are used in clinical biochemistry laboratory; which prove efficient in various clinical conditions.

The formation of AOPP and IMA is very well correlated with the increase in free radicals. There are reports stating the increase in the levels of modified protein products (such as AOPP and IMA) in particular situations where there is an increase in the levels of free radicals (Borderie et al., 2004). With such a relation established between AOPP and IMA, their relation with increases in the lipid profile levels suggests the involvement of these risk factors pointing towards cardiovascular diseases. The significant increase in the lipid profile levels in line with the increase in AOPP levels and IMA levels shows that the risk factors involved in cardiovascular diseases have a link to the formation of AOPP and IMA. With the increase in the levels of free radicals, there is an increase seen in the levels of AOPP and IMA and in turn this combined with the increase in the levels of risk factors which are said to be involved in cardiovascular diseases, it all turns to the increase in the chances of the latter occurring in such a condition.

The ability of albumin to bind to various ligands makes it ideal for acting as a buffering agent to some endogenous and exogenous molecules that are toxic in nature. In some populations, IMA was primarily considered for use as a diagnostic marker for acute coronary syndromes (ACS) (Peacock et al., 2006; Sinha et al., 2003). The increased sensitivity shown by IMA is at the level of detecting myocardial ischemia at subclinical levels (Van Belle et al., 2010). Any increase in the levels of IMA would indicate a reduction in the binding capacity of albumin to metals, which is associated with cardiac ischemia and also indicative of the oxygen free radical species formation (Marx et al., 1986). The capacity of human serum albumin to bind to particular metal ions (transitional) is the property by which IMA acts as a marker of ischemia. This is in particular to the N-terminus and the metal ions such as cobalt and copper bind to this terminus. According to a report by Bar-Or, in conditions where albumin is exposed to the changes which occur in ischemic tissue, the N-terminus loses the ability to bind to metal ions due to the changes that take place in the human serum albumin (Bar-Or et al., 2001; Bar-Or et al., 2000). There is also an increase of IMA shown in conditions with increased ROS generation. An increase in IMA levels was also observed in a case after minutes of transient occlusion and reperfusion during coronary angioplasty.

The importance of AOPP and IMA in relation to markers in cardio-vascular pathology, the ability of their use in as cardiac markers is reported in the
literature. The results from our study state the relationship between AOPP, IMA with the basic biochemical parameters such as lipid profile, BMI etc. It was seen that there were particular significant correlations between some of the basic parameters with AOPP and IMA. This relationship could possibly be the first step in exploring the relationship between AOPP and IMA with the risk factors analyzed in our study. This, if supported with more evidence by relating and supporting their correlations in different conditions, could probably prove a simple and cost effective analysis in the primitive analysis of ACS.

**Conclusion**

This study shows the possible direct correlation of AOPP levels to those of BMI, levels of LDL, TG, TCHOL, TCHOL/HDL, and IMA. AOPP or the advanced oxidation protein products are the modified proteins that might have a positive correlation with LDL, TG and other parameters used in the study. This shows that accumulation of AOPP or the formation of the same could relatively increase the levels of LDL and could indirectly lead to cardiac disorders. Such understandings with respect to AOPP could possibly enhance the understanding of the role of AOPP internally. These studies also provide a better understanding of the role of these AOPPs formed in various disease conditions. Oxidative stress induced protein modification product AOPP had direct correlation with risk factors of cardiovascular risk factors such as BMI, LDL, Total Cholesterol across the group. IMA the established marker of myocardial ischemia on the other hand, does not show the same correlation with the risk factors as well as AOPP indicating that mechanism of formation of AOPP may be different from that of IMA. Between the groups with difference in BP, AOPP and IMA have no much difference in correlation with risk factors. Limitation of this study is that groups divided based on BP contain the other risk factors used which might also influence the level of AOPP and IMA. Because of the highly prevalent presence of risk factors of cardiovascular diseases, in order to study the correlation of individual risk factor with IMA and AOPP, it is important to carry out studies with a large sample size.

**Recommendations**

Such studies, if carried out with a large sample size would provide more clarity regarding the significant correlations between the various parameters.

**Funding and Policy Aspects**

The authors feel that such studies linking the different aspects of even the well known diseases and the factors linking to smaller contributions from other parameters should be encouraged by the funding agencies to get a better understanding of such diseases.

**Authors’ Contribution and Competing Interests**

Kundapura Arun Kumar: Designed the study, wrote the protocol, and performed the analysis and literature search.

Sheila Uthappa: Guided in designing the study and writing the protocol

Sudarshan Surendran: Literature search and wrote the first draft of the manuscript

Avinash S.S.: Helped in analysis

Sucharitha Suresh: Helped in analysis

Sushitha E.S.: Performed the statistical analysis

Authors have declared that no competing interests exist.

**Acknowledgement**

The authors wish to acknowledge the support and encouragement by the management of Father Muller Medical College. We are grateful to Rev. Fr. Patrick Rodrigues, Director; Rev. Fr. Ravi Rudolph D’Sa, Administrator; Dr. JP Alva, Dean, and Dr. Malathi M., Professor and Head of Biochemistry.

**References**


Apple F.S., Wu A.H., Mair J., Ravkilde J., Panteghini M., Tate J., Pagani F., Christenson...


Rudling M., 2006. [Lowering of LDL cholesterol prevents cardiovascular diseases. "Normal values" are too high--treatment time is a crucial factor]. Lakartidningen 103(43), 3278-82.


