

# Microalbuminuria in Angiographically Documented Coronary Heart Disease in Non Diabetic and Normotensive Individuals

ZUBAIR A A.,<sup>1</sup> ABDUL K N.,<sup>2</sup> MAUJID M M.,<sup>3</sup> SHAKIR K.<sup>4</sup>

*Address for Correspondence:* Brig Abdul Khaliq Naveed Dean, Prof & Head Department of Biochemistry and Molecular Biology, Army Medical College, Rawalpindi

**Background:** Coronary heart disease (CHD) is the most prevalent and important cause of death in developed nations. The incidence of this disease has also increased in our country during the last few years. This occurs due to atherosclerotic narrowing of coronary arteries, mainly because of hypercholesterolemia, hypertension, diabetes mellitus, physical inactivity and cigarette smoking. CHD manifests as stable angina (SA), unstable angina (UA) and myocardial infarction (MI). In spite of the curtailment of these traditional risk factors the disease has not been eliminated from the globe so far. Recent research in the developed countries is focusing now to identify new biomarkers associated with CHD. Microalbuminuria (MA) has implications on the development of CHD and it is emerging as a new risk factor of this disease.

**Objectives:** To compare the levels of microalbuminuria in CHD patients and control individuals with aim to evaluate its association with CHD.

**Study Design:** A case control analytical study conducted at Army Medical College and Armed Forces Institute of Cardiology Rawalpindi.

**Material and Methods:** Thirty controls (groups A) and fifty CHD patients (group B) included in this study were non diabetic and non hypertensive. Microalbuminuria was determined by immunoturbidimetric method. Serum glucose, cholesterol, triacylglycerol and urea were measured by enzymatic method. Serum creatinine was measured by kinetic colorimetric method.

**Results:** Microalbuminuria, cholesterol and triacylglycerol of group B were compared with group A. A significant difference was found with p value < 0.05.

**Conclusion:** Microalbuminuria may have an association with coronary heart disease.

**Key Words:** Coronary heart disease (CHD), stable angina (SA), unstable angina (UA), myocardial infarction (MI), Microalbuminuria (MA)

## Introduction

The terms coronary artery disease (CAD), ischaemia heart disease (IHD) and coronary heart disease (CHD) are synonymous but the common form of heart disease is CHD. This is always due to atherosclerosis of coronary arteries long before it manifests as angina pectoris, unstable angina, myocardial infarction and chronic IHD with heart failure.<sup>1</sup> Despite of adopting, the preventive measures and the treatment against established risk factors i.e. smoking, obesity, physical inactivity, diet, hypercholesterolemia, diabetes mellitus and hypertension, the incidence of the CHD is still on rise. It is expected that CHD will become the most common cause of death in human history all over the world by the year 2020.<sup>2</sup> At present the countries like India, Pakistan, Bangladesh, Sri Lanka and Nepal has the highest incidence of CHD when compared globally. Moreover most of the studies on CHD has been conducted in Bangladesh, India and Pakistan.<sup>3,4</sup> Comprehensive research in the field has emerged with multiple new biomarkers and inflammatory markers of CHD such as, increased lipoproteins (a) levels, total plasma homocysteine, elevated plasma fibrinogen levels, plasminogen activating inhibitor (PAI), C-reactive protein (CRP), different cytokins and microalbuminuria. The effect

of risk factors is multiplicative rather than additive. Individuals with a multiple risk factors are at more risk, so assessment should be based on a holistic approach that should account all identifiable risk factors.<sup>5,6</sup>

Accumulative evidence suggests that the pathological changes causing microalbuminuria and the pathological changes leading to premature atherosclerosis are the same. Decreased density of heparin sulphate in the endothelial cells of vessels, leads to decreased binding of lipoprotein lipase, which results into decreased clearance of very low density lipoproteins and leads to hyperlipidemia. Similarly endothelial dysfunction, the initial stage of atherosclerosis and low grade inflammation is common between MA and cardiovascular disease.<sup>7,8</sup>

Albuminuria is normally diagnosed with strips such as albutix, which corresponds to urinary albumin excretion rate (UAER) > 300 mg/ 24 hour. Microalbuminuria is defined as the UAER between 30- 300 mg / 24 hour.<sup>9</sup>

Microalbuminuria (MA) as a biomarker now a days is also considered a risk marker for CHD in diabetics and non diabetics. Patients with microalbuminuria and concomitant diabetes have higher rate of mortality due to the develop-

ment of CHD. In clinically healthy subjects the levels of atherogenic risk factors are increased if they have associated problem of microalbuminuria. It is also noticed that the patients with MA have more severe angiographic CAD than those without MA (10). There is increased atherogenic risk factor pattern even in normotensive persons with MA. So it may be taken as a marker for CHD in such patients although it is not certain that the associated metabolic changes of atherosclerosis are due to MA or results from some other metabolic disturbances such as insulin resistance.<sup>11</sup> Moreover MA is independently associated with cardiovascular morbidity, after adjusting the known cardiovascular risk factors of the prevalence of CVD in men and women. It may be a useful indicator of an absolute high cardiovascular risk in the community; yet prospective data is needed to establish its independent predictive value for future events.<sup>12</sup> Where persistent microalbuminuria in diabetic patients is considered a risk factor for renal disease, there it can also be the cause of morbidity and death. Even microalbuminuric individuals without diabetes tend to have an increased cardiovascular morbidity in the heart patients. Furthermore the established cardiovascular risk factors are more frequent in diabetic individuals with persistent MA than in normoalbuminuric diabetic individuals of the same age and sex. About 50% of the albuminuric patients will die due to cardiovascular causes well before the development of the progressing end stage renal disease. Even in non diabetic patients, MA has been noticed as factor for the premature atherosclerosis.<sup>13</sup>

Among elderly people aged 60-74 years having MA, the associated cardiovascular disease was found as the most frequent cause of mortality even when established risk factors were taken into account for CVD. Similarly in a cross sectional community survey MA was uncommon in general population and was related to ageing, blood pressure and the other cardiovascular risk factors. It may reflect the presence of already existing cardiovascular disease.<sup>14,15</sup> Prothrombotic state has no interrelationship between microalbuminuria and atherosclerotic vascular chain and this fact is verified by the presence of unaltered haemostatic balance in healthy subjects with microalbuminuria.

These findings support the idea that the MA alone may act as risk for atherosclerosis.<sup>16</sup> A cross sectional cohort study stated that an increased urinary albumin excretion rate was related with CVD, dyslipidemia and hypertension. It indicates that MA was not independent predictor of CHD but markedly enhanced the risk, along with other established risk factors.<sup>17</sup> In patients of type 1 diabetes mellitus the possible effect of MA does not depend on gender, age, hypertension, smoking, total cholesterol, HDL cholesterol and other risk factors. Mild albuminuria not only depends upon pressure effect in blood vessels of glomeruli, but also signifies atherosclerosis related disorders in the whole vascular system.<sup>18,19</sup>

It is also noted that the MA and peripheral arterial disease (PAD) are related with an enhanced number of deaths in

heart diseases and are mutually independent risk indicators, where MA affects mortality risk through a mechanism different from generalized atherosclerosis.<sup>20</sup> CAD coexisting with MA is the main cause of morbidity and mortality in patients with PAD. Clinical evaluation and non-invasive test have limitations for the detection of CAD in patients with PAD.<sup>21</sup> Furthermore MA is the early detector of death and morbidity in patients suffering from diabetes and hypertension which confirmed the involvement of albuminuria as risk factor for deaths due to heart diseases in future, which is independent of hypertension and diabetes mellitus.<sup>22</sup> In a prospective study non diabetic and hypertensive patients were screened for MA by reagent strips which revealed that MA identifies hypertensive patients, particularly at risk of cardiovascular disease.<sup>23</sup>

High sensitivity C-reactive protein has also been found raised in the serum of general population having microalbuminuria, marking microalbuminuria as useful indicator representing low grade systemic inflammation and also a risk factor for cardiovascular system in apparently healthy individuals.<sup>24</sup> The frequency of MA has been found 37% in non diabetic patients and it was highest in elderly patients.<sup>25</sup> Keeping in view the present perspective this study has been conducted to evaluate the association of microalbuminuria in our population.

### **Aim and Objectives**

This study has been conducted to compare the levels of microalbuminuria in normal individuals and CHD patients with the aim to evaluate their association with coronary heart disease.

### **Material and Methods**

#### **Setting**

The study has been conducted in the Department of Biochemistry and Molecular Biology Army Medical College Rawalpindi. Patients and control individuals were selected from Armed Forces Institute of Cardiology (AFIC) Rawalpindi. Laboratory analysis was done in the Department of Chemical Pathology, Army Medical College Rawalpindi.

#### **Study Design**

This was a case control analytical study conducted on eighty individuals comprised of two groups who were selected by convenient sampling from AFIC.

**Group A (Controls).** Thirty individuals having normal ECG, ETT and cardiac enzymes. All these individuals were having normal coronary angiograms.

**Group B (Patients).** Comprised of fifty individuals and having CHD on the basis of abnormal coronary angiogram with involvement of single vessel, or double vessel, or triple vessel coronary disease.

#### **Inclusion Criteria**

All individuals were normotensive and non diabetic.

### Exclusion Criteria

All Individuals with renal diseases, arthritis, pyrexia, hepatitis, malignancy and acute or chronic inflammatory conditions.

### Methods

Venous blood sample was collected after over night fasting of 10-12 hrs. Two ml blood was transferred to test tube containing potassium fluoride for serum glucose assay after centrifugation. Rest of the blood was allowed to coagulate and centrifuged. Serum was stored at -40°C. First morning urine samples were collected and stored at -40 °C.

Serum glucose,<sup>26</sup> triacylglycerol<sup>27</sup> cholesterol,<sup>28</sup> and urea<sup>29</sup> levels were determined by using enzymatic method while the serum and urinary creatinine concentrations were determined by kinetic colorimetric method.<sup>30</sup> Microalbuminuria was measured by immunoturbidimetric assay.<sup>31</sup> The ratio of albumin (µgm) and Creatinine (mg) (ACR) was calculated to determine microalbuminuria.

### Statistical Analysis

All values were calculated as mean ± standard error mean and the comparisons were made between both groups A&B by applying student's t test. The differences were taken as statistically significant when *p* value < 0.05. Computer programme SPSS 14.0 was used for statistical analysis.

### Results

Individuals of both groups were non hypertensive and non diabetic confirmed on biochemical evaluation. This evaluation was done by performing various biochemical tests (fasting glucose, urea and Creatinine) in both groups. The levels of microalbuminuria, serum triacylglycerol and cholesterol were measured in both groups. Age, blood pressure and body weight were also recorded and noted in both groups.

#### Group A (Control) and Group B (Patients)

Levels of microalbuminuria (ACR) in groups A & B were

**Table: 1** Comparison of levels of microalbuminuria and serum levels of total cholesterol and triacylglycerol in Group-A and Group-B. The values are expressed in mean ± SEM. The number of observations is given in parenthesis.

Study group	Microalbuminuria (µg/mg)	Total cholesterol (mg/dl)	Triacylglycerol (mg/dl)
Group – A (30)	21.78 ± 1.01	176.80 ± 4.57	196.70 ± 9.06
Group – B (50)	*36.58 ± 3.78	*235.56 ± 5.32	*245.32 ± 8.16

\**P* < 0.05. The values in group B are significantly different as compared to group A.

21.78 µg/mg ± 1.01 and 36.58 µg/mg ± 3.78 respectively. Total cholesterol values in groups A & B were 176.80 mg/dl ± 4.57 and 235.56 mg/dl ± 5.32 respectively. Triacylglycerol values in groups A & B were 196.70 mg/dl ± 9.06 and 245.32 mg/dl ± 8.16 respectively (Table-1). All these values are different significantly (*P* < 0.05) when both groups were compared with each other.

Fasting glucose values of the individuals of group A & B were 85.53 mg /dl ± 1.51 and 88.98 mg/dl ± 1.29 respectively. Serum creatinine values in groups A & B were 0.89 mg/dl ± 0.02 and 0.93 mg/dl ± 0.02 respectively. Serum urea values in both groups A & B were 29.50 mg/dl ± 1.22 and 28.80 mg/dl ± 1.13 respectively (Table 2). No statistically significant difference was found (*P* value > 0.05) in glucose, creatinine and urea values between both groups A&B.

Mean age (years) of groups A & B was 44.6 and 46.7 respectively. Mean bodyweight (kg) of groups A & B was 70.96 and 72.78 respectively. Mean values of systolic and diastolic blood pressure of groups A & B were 120/79 mm Hg and 121/79 mmHg respectively. The above mentioned values of group A were compared with the respective values of group B. *P* value (*p* > 0.05) showed no significant difference in mean values of age, body weight and blood pressure of groups A & B.

### Discussion

The pandemic of CVD appeared in the beginning of the 20<sup>th</sup> century as a 4<sup>th</sup> most frequent cause of mortality in the developed countries, which acquired first place by the end of first decade. Even though much has been discerned about the causes of CHD but still 50% of CHD patients do not have any of already known risk factors i.e blood pressure, raised cholesterol, diabetes, marked obesity and sedentary life pattern. Moreover death due to CHD has increased much more in developing countries (32). In spite of the changes in life style and application of adequate therapeutic measures to lower down the cholesterol levels, CHD continues to be the major cause of morbidity and mortality.

Cholesterol screening fails to recognize almost 50% of those individuals who present with acute coronary syndrome (ACS).<sup>33</sup>

Epidemiologic and experimental data shows that microalbuminuria is associated with high incidence and increased risk for cardiovascular mortality among individuals with diabetes and hypertension. The risk for cardiovascular events increases even in healthy non-hypertensives and non-diabetics who have microalbuminuria below the normal threshold level.<sup>34</sup>

Inadequate studies relating to microalbuminuria are available in literature particularly when study population under investigation is of non hypertensive and non diabetic patients. No studies have been carried out on the above-mentioned population in Pakistan. In our study population the results of microalbuminuria of group-A (control) and group B (patients) are presented here for discussion and comparison with other available data.

In group-A values of microalbuminuria (ACR), were 21.78 ug/mg  $\pm$  1.01. In group-B values of microalbuminuria (ACR) were 36.58 ug/mg  $\pm$  3.78. When we compared the values of microalbuminuria of group-A with the group-B a significant difference was found with  $p < 0.05$  (Table-1).

When these results were compared with a population based prospective study, it was revealed that microalbuminuria is a potent and clinically relevant risk marker for the development of IHD and also predicts IHD independently of other classic atherosclerotic risk factors.<sup>17</sup> Similarly the risk of CHD in relation to hyperinsulinemia and microalbuminuria increases in patients with prevalence of other adverse cardiovascular risk factors. So the investigation of the mechanisms contributing to the high CHD risk in individuals with MA could be a challenge for future studies.<sup>35</sup> In another study MA conferred a four fold increased risk of IHD among hypertensive or borderline hypertensive individuals.<sup>36</sup>

Furthermore a cross sectional study conducted on non diabetic individuals showed that there was an independent association between MA and ischaemic electrocardiographic abnormalities which supports that MA has an additional value to conventional risk indicators for predicting CVD.<sup>37</sup> The results of the above mentioned studies are consistent with and support our findings that low-grade microalbuminuria is associated with CHD in conjunction with hypertension or diabetes and also in individual without high blood pressure and diabetes. Also results of our study are consistent with a population based study of non diabetic individuals which showed that even small amount of albumin excreted in the urine is associated with increased risk of CHD. Same study has also addressed the effect of MA as cardiovascular risk in non diabetics.<sup>12</sup> A study carried out by Arnlov J et al showed the association of MA in 1568 nonhypertensive and non diabetic individuals with the incidence of CVD events, and reported that low levels of urinary albumin excretion well below current threshold of microalbuminuria predicts the development of CVD and supports the notion that albuminuria of less intensity can be a marker, indicating damage to vessels subclinically and subjects the patients to CVD and death in future.<sup>38</sup> MA is also well known risk factor for CHD in diabetics but non diabetic CHD patients with MA have more severe CAD than those individuals without MA which indicates that MA enhances the chance of development of CHD.<sup>10</sup> In a study conducted on Pakistani population, the occurrence of MA was more common in

non diabetic CHD patients than in the general population which is consistent with our study.<sup>25</sup>

**Table 2:** Serum values of fasting glucose, creatinine and urea in group-A and group B. The values are expressed as mean  $\pm$  SEM. The number of observations is given in parenthesis.

Study group	Fasting glucose (mg/dl)	Creatinine (mg/dl)	Urea (mg/dl)
Group – A (30)	85.53 $\pm$ 1.51	0.89 $\pm$ 0.02	29.50 $\pm$ 1.22
Group – B (50)	88.98 $\pm$ 1.29	0.93 $\pm$ 0.02	28.80 $\pm$ 1.13

No statistically significant difference ( $P > 0.05$ ) between the values of both groups B & A.

In a prospective cohort study it was observed that MA was associated with prevalence of CHD and subsequent mortality in those South Asians men living in UK. This suggests that CHD risk differs by ethnicity.<sup>39</sup>

Above mentioned perspective data evaluating the microalbuminuria and its association with CHD in non-hypertensive and non diabetic individuals is sparse and our study is consistent with this data.

When we compared the values of total cholesterol and triacylglycerol between both groups A&B a significant difference ( $p < 0.05$ ) was found (Table 1). This result is consistent with the studies, in which lipid levels were found raised in conjunction with microalbuminuria.<sup>10,11,13</sup>

From the above mentioned discussion it is evident that microalbuminuria not only identifies the individuals at risk but it can be a contributory factor in the development of CHD and its progression. However, large group prospective cohort studies are required to establish relationship between this marker and disease.

## Conclusion

Raised levels of the microalbuminuria, in non diabetic and non hypertensive CHD patients may have an association with coronary heart disease.

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