Recent advancements regarding Vitamin D3

The endocrine system of vitamin D is central to the control of bone and calcium homeostasis. D2 and D3 are the two major types of Vitamin D. Vitamin D2 is synthesized in plants and fungi, whereas Vitamin D3 or calcitriol is produced in relatively large amounts in humans and in majority of vertebrate animals. The active metabolite of Vitamin D, 1α, 25-hydroxyvitamin D3 [1, 25 (OH) 2D3] is involved in calcium and phosphate metabolism and exert a large no of biological effects. Vitamin D inhibits parathyroid hormone secretion, adaptive immunity and cell proliferation and at the same time promotes insulin secretion, innate immunity and stimulates cellular differentiation. The role of Vitamin D in immune-regulation has led to the concept of dual function as both as an important secosteroid hormone for the regulation of body calcium homeostasis and as essential organic compound that has been shown to have a crucial effects on the immune responses. Altered levels of vitamin D3 have been associated, by recent observation studies, with a higher susceptibility of immune-mediated disorders and inflammatory diseases.

Vitamin D3 is photosynthesized in the skin, in which UV light catalyzes the first step in D3 biosynthesis. The key step of Vitamin D biosynthesis involves different isoforms of cytochrome P450 family. Following biosynthesis, inactive vitamin D3 or calcidiol is transported to the proximal tubule of kidney, where it is hydrolyzed to active form.

Significant advancements are made from different studies in defining the role of Vitamin D in innate immunity. Vitamin D regulates the production of T-cell helper 1 (Th1) and Th2 cytokines and 1L-17, by which it influences adaptive immunity and inflammation. Interestingly, specific T-cell cytokines are able to influence the TLR induced vitamin-D-dependent anti-microbial pathway in human monocytes. The Th1 cytokine IFN-γ enhances the TLR2/1 induction of CYP27B1, cathelicidin and DFEB4. Recent studies have shown a marked effect of Vitamin D3 in activity of cytokine like TNF-α expression in inflammatory disease. The down regulatory effect of D3 is not unique to TNF-α expression and was found to decrease the synthesis of IL-2 in activated lymphocytes and also inhibited expression of 1L-1, IL-6 and IL-12 in cultured monocytes and macrophages. The ability of vitamin D3 to suppress mycobacterium TB growth in monocytes has also been linked to the production of bactericidal superoxide anions and to the generation of phagocyte derived reactive oxygen synthesis (NO).
Vitamin D acts through its receptor VDR, which upon binding to vitamin D3 gets activated and regulates transcription of different classes of genes including those involved in immune reaction. Vitamin D receptor or VDR is one of the DNA-binding transcription factor but has an important additional property, which it shares only with some other members of nuclear receptor superfamily. VDR can get specifically activated by macromolecular concentrations of lipophilic molecule, the property which is shared with nuclear receptor nuclear receptor for steroid hormones. Like other members of the human nuclear receptor superfamily, VDR is characterized by a highly conserved DNA binding domain (DBD) and a structurally conserved ligand-binding domain (LBD). As observed with other transcription factors, the DBD and of VDR cannot contact more than six nucleotides within the major groove of the target DNA and the conserved sequence for VDR binding is RGKTS (R= A or G, K= G or T and S= C or G), which is also referred to as VDRE or vitamin D response element. Several recent studies using chromatin immune precipitation assay or ChIP technique have shown there are number of genes that are regulated by VDR. Vitamin D3 stimulation of lymphoblastoma or human monocyte has shown to trigger VDR binding to more than 1500 sites within the genome. The VDR binding site of this gene is located within the intron some 110kb downstream of TSS.

Number of recent studies suggests that VDR maturation is associated with number of diseases mainly due to defects in immunomodulation. VDR is suggested to play important role in miRNA regulation and thus might indirectly involved in key regulation of other genes. VDR also regulates MCM7 gene that encodes MIR 106b and known to regulate expressions of MIR181a and MIR-22 (14,15). Micro RNA regulation is linked to several diseases and their presence sometimes used as marker for some diseases.

RFLP studies from different segments of VDR gene demonstrated polymorphism in the gene and mutations in the VDR gene are associated with different disease progression among individuals. These diseases include diabetes, tuberculosis, leprosy, cancer and bone biology which are directly or indirectly associated with vitamin D levels. Analysis of the TaqI polymorphism in the 3’ region of the VDR gene showed that the overall distributions of genotypes between the groups studied were different. In tuberculoid leprosy, the tt genotype was found at significantly higher frequency than in the controls. In contrast, the TT genotype was found at increased frequency in the lepromatous leprosy group compared with the controls. Taken together, all these information clearly suggesting vitamin D and VDR together plays a very significant role in innate and adaptive immunity that controls number of human diseases and new drug regimen must include vitamin D supplement and alternatively VDR therapy might be helpful.
Introduction

Sickle cell disease (SCD) is a monogenetic recessive haemoglobinopathy that affects millions throughout the world. It is common among people whose ancestors come from Sub-Saharan Africa, South America, Cuba, Central America, Saudi Arabia, India, and Mediterranean countries. SCD is prevalent in India especially in states of Chhattisgarh, Odisha, Maharashtra, Telangana and Gujarat among others.(1) As per ongoing studies, SCD gene affects about 25 lac people that constitute about 10% of the Chhattisgarh state’s population.(2) Heterozygous state (AS) of this gene referred as sickle cell trait, is asymptomatic, but homozygous state (SS) of this gene causes SCD. This disease is a significant cause of morbidity and mortality in the state. SCD is caused by a point mutation (GAG → GTG) in the beta globin gene (11p15.5) leading to replacement of the polar amino acid glutamic acid with a hydrophobic amino acid valine at the 6th position in beta globin chain of haemoglobin molecule.(3) These mutant beta globin chains (βS) in homozygous state lead to the formation of haemoglobin S (HbS), which gets polymerized through hydrophobic interactions under hypoxic conditions, to form a rope like fiber composed of seven double strands twisted helically around a vertical axis.(4) Depending on their orientation within the
erythrocyte, these polymers deform the shape of the cell to produce one of several morphological forms, mainly sickle shaped, instead of normal biconcave disc shape. Initially the episodes of sickling are reversible but repeated episodes of sickling damage the erythrocyte cell membrane and decrease the cell’s elasticity. Such erythrocytes fail to return to their normal shape, even under normal oxygen tension. Further, activity of erythrocyte membrane Na⁺ K⁺ ATPase is increased in SCD patients in comparison to normal (AA) and sickle cell trait (AS) individuals. The specific ratio of Na⁺ to K⁺ transportation through Na⁺ K⁺ATPase on red blood cell membrane is 3:2. Thus increased activity of Na⁺ K⁺ATPase on RBC membrane leads to disturbed internal ionic milieu of RBCs, which leads to cellular dehydration and distortion of RBC shape and volume.

Till date no pharmaco-therapeutic cure is available for this disease. Only disease modifying therapies available till date are blood transfusion and Hydroxyurea. Suggested mechanism of Hydroxyurea is elevation of protective HbF level in RBCs. In this study, we have tried to analyze the effect of Hydroxyurea therapy on HbF level, Na⁺ K⁺ATPase activity and other haematological parameters and deduce other possible mechanisms involved in the therapeutic effect of Hydroxyurea on SCD patients.

**Materials and Methods**

This study was done in the department of Biochemistry, Pt. J.N.M. Medical College Raipur after getting approval by the institutional ethics committee.

**Recruitment of subjects**

The study group comprised of 80 subjects. The group was divided into three subgroups viz., Normal Controls AA (20 subjects), Sickle Cell trait AS individuals (20 subjects) and SCD patients (40 subjects). SCD patients’ subgroup comprised of further two subgroups of 20 subjects each, viz., those on Hydroxyurea therapy (SCD Hyd+) and those not on Hydroxyurea therapy (SCD Hyd-). The mean age in the AA, AS, SCD Hyd+ and SCD Hyd- groups were 17 ± 3.2, 15.15 ± 1.87, 14.65 ± 2.3 and 14.55 ± 2.32 years respectively. Differences in the mean to female ratio in the study groups were also not statistically significant. Initial identification of disease status of subjects was done using haemoglobin solubility test. Subjects showing negative solubility test results, were identified as Normal controls. Those showing positive solubility test results, were subjected to haemoglobin electrophoresis in alkaline medium to identify their SCD status. This electrophoresis step was used to discriminate Sickle Cell trait individuals and SCD patients. Identified SCD patients were subjected to High Performance Liquid Chromatography (HPLC) to estimate the content of Foetal haemoglobin in SCD patients. Patients in Hyd+ group were recruited only if they were on Hydroxyurea therapy for at least one month. The dose being administered to them was 10 mg/kg body weight alternate day. Patients in Hyd- group were those, who had no history of Hydroxyurea therapy. Patients with any other associated haemoglobinopathy or blood dyscrasias, history of discontinued Hydroxyurea therapy, severe bone marrow depression, liver & kidney disorders, other chronic debilitating diseases were excluded from this study. Moreover pregnant & lactating women and malnourished children were also excluded.

**Sample Collection and laboratory analysis**

After getting written informed consent, five milliliter of venous blood was collected from subjects in vials containing 0.05 ml of EDTA as anticoagulant. Subjects were identified as sickle cell trait (AS) and SCD (SS) through electrophoresis. For this purpose, about 300 μl of blood sample was used for alkaline paper electrophoresis for subjects with positive solubility test. 5 μl of EDTA added blood samples of AS and SS subjects were used to measure fetal
haemoglobin level. For this purpose Biorad Hb Variants HPLC analyzer was used. Other hematological parameters viz., haemoglobin level, RBC count, total leucocyte count (TLC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), were measured by using automated hematology cell counter analyzer (Mindray BD – 300 plus). Erythrocyte membrane Na+ K+ ATPase activity was measured in the form of released of inorganic phosphate (Pi) per mg of protein & expressed as (μmol Pi/mg protein/hr) by the action of Phosphatases present in the RBC membrane(7). The phosphate concentration was measured by using the standard curve made using Fiske & Subbarow principle and the membrane protein was quantified by using Lowry’s principle(11). For measuring total bilirubin, blood was taken without adding anticoagulant and bilirubin was measured by I Lab 650 autoanalyzer. (Fig. 1)

**Statistical Analysis**

Statistical analysis was done using SPSS version 13. Data was presented as mean and standard deviation (SD). Unpaired Student’s t-test, ANOVA and Post – hoc Bonferroni test were used to analyze the observed data. A P value <0.05 was considered statistically significant.

**Results**

Na+ K+ ATPase activity in RBC membrane:

Mean Na+ K+ ATPase activity in terms of μmol Pi/mg protein/hr, AA, AS, SCD Hyd- and SCD Hyd+ subgroups is depicted in Fig. 2. The Na+-K+ATPase activity in the AA, AS, SCD Hyd- and SCD Hyd+ subgroups was found to be 104.52±18.18, 117.93±19.15, 153.60±5.76, 135.29±24.30 μmol pi/mg protein/hr respectively. Applying ANOVA the difference between four groups was found to be significant (F=23.629, p<0.0001). The Post – Hoc Bonferroni test was used to estimate the significance of pair-wise difference of Na+ K+ ATPase activity in terms of μmol Pi/mg protein/hr, in AA, AS, SCD Hyd- and SCD Hyd+ subgroups. The results obtained are depicted in Table 1. Difference between AA and AS groups was not found to be statistically significant. While other groups including SCD Hyd- vs SCD Hyd+ had statistically significant difference in Na+ K+ ATPase activity. (Fig. 2)

**Foetal Haemoglobin Levels in RBCs**

The HbF level in the AA, AS, SCD Hyd- and SCD Hyd+ subgroups is depicted in Fig. 3. HbF level in the AA, AS, SCD Hyd- and SCD Hyd+ subgroups was found to be 0.58±0.28%, 2.55±1.78%, 18.07±3.9% and 22.61±6.22% respectively. Applying ANOVA the difference between four groups was found to be significant (F=170.208, p<0.0001). Difference between AA and AS groups was not found to be statistically significant. While other groups including SCD Hyd- vs SCD Hyd+ had statistically significant difference in HbF level. (Fig. 3).

Results of comparision of Na+ K+ ATPase activity and HbF level are provided in Table 1.

**Haematological and other parameters**

Detailed values of haematological and other parameters are given in Table 2. Applying unpaired student t test the difference of haemoglobin concentration between these two groups was found to be statistically significant (p<0.0001). Applying ANOVA the difference in haemoglobin concentration between three groups was found to be significant (F=21.11, p<0.0001). The Post – Hoc Bonferroni test showed that the SS patients had a significantly lower total RBC count then the AA & AS subjects. Difference in RBC count between the AA & AS groups was not found to be statistically significant (p value>0.05). Similarly, mean RBC count SCD Hyd+ group was found to be significantly higher SCD Hyd- groups (p<0.01). Applying ANOVA the difference in the TLC between three groups was found to be
statistically significant (F=7.652, p<0.001). The Post–Hoc Bonferroni test showed that the SCD patients had a significantly higher TLC than the AA and AS subjects. But the difference between the AA & AS groups was not found to be statistically significant. There was no significant difference in TLC count between SCD Hyd+ group & SCD Hyd- groups (p=0.086).

Applying ANOVA the difference in MCV between four groups was found to be significant (F=3.680, p=0.016). The Post–Hoc Bonferroni test showed that the difference in MCV values between AA–AS, AA–SCD Hyd- and AA– SCD Hyd+ groups was statistically significant (p<0.01). On the other hand difference in MCV values between AS– SCD Hyd-, AS–SCD Hyd+ and SCD Hyd– SCD Hyd+ groups was not statistically significant (p>0.05).

Applying ANOVA the difference between the values of MCH for four groups was found to be statistically significant (F=3.964, p=0.011). The Post–Hoc Bonferroni test showed that the difference in the values of MCH between AA– SCD Hyd- group was statistically significant (p<0.01). But AA–AS , AA–SCD Hyd+, AS - SCD Hyd-, AS - SCD Hyd+ & SCD Hyd– SCD Hyd+ groups was not statistically significant (p>0.05).

Applying ANOVA the difference between the values of MCHC for four groups was found to be significant (F=2.810, p=0.045). The Post–Hoc Bonferroni test shows the difference in the values of MCHC between AS – SCD Hyd- was statistically significant (p<0.05) but AA – AS , AA – SCD Hyd-, AA – SCD Hyd+ , AS - SCD Hyd+ & SCD Hyd– SCD Hyd+ groups was not statistically significant (p>0.05).

Another interesting finding was significant difference in total serum bilirubin level between SCD Hyd- and SCD Hyd+ groups (p<0.0001). Applying ANOVA the difference of total serum bilirubin concentration between three groups was found to be significant (F=21.766, p<0.0001).

Discussion
This study was undertaken to determine the alteration in erythrocyte membrane Na⁺-K⁺ ATPase activity, HbF level and haematological and other parameters in sickle cell disease patients under Hydroxyurea therapy. This study has revealed that the increased Na⁺-K⁺ ATPase activity in SCD patients may be decreased to normalcy by the use of Hydroxyurea therapy. This may be an additional mechanism of Hydroxyurea, in treatment of SCD patients in addition to increasing HbF levels. Thus Hydroxyurea may be helpful in maintaining the internal ionic milieu of RBCs and thus indirectly the shape and volume of RBCs. As evident from several studies, this study also has confirmed that Hydroxyurea increases the HbF level inside RBCs in SCD patients(12). As far as use of Hydroxyurea is concerned, this study has shown that Hydroxyurea improves RBC count in SCD patients. Total serum bilirubin level also has shown significant improvement upon use of Hydroxyurea.

Other parameters including TLC, MCV, MCH, MCHC have not shown any significant difference between the groups on therapy and those not on Hydroxyurea therapy. As the size of sample included in this study was small, the findings may be validated using a larger data set.

Conclusion
This study showed that the increased Na⁺ K⁺ ATPase activity in SCD patients may be decreased to normal level by the use of Hydroxyurea therapy. This effect may be an additional mechanism of Hydroxyurea, in treatment of SCD patients in addition to increasing HbF levels. Further studies may be undertaken with larger data sets to validate the findings in this study.
Hydroxyurea therapy & Biochemical parameters in sickle cell disease

Fig. 1 : Standards and standard curve for phosphate estimation

Fig. 2 : Mean values of Na⁺ K⁺ ATPase activity in RBC membrane

Fig. 3 : Mean values of foetal haemoglobin (HbF)
Table 1: Statistical significance of difference in $\text{Na}^+ \text{ K}^+ \text{ ATPase}$ activity and HbF level

<table>
<thead>
<tr>
<th>Comparison groups</th>
<th>$p$ value for difference in $\text{Na}^+ \text{ K}^+ \text{ ATPase}$ activity</th>
<th>$p$ value for difference in HbF level</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA – AS</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>AA – SCD Hyd-</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>AA – SCD Hyd+</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>AS – SCD Hyd-</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>AS – SCD Hyd+</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>SCD Hyd- – SCD Hyd+</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

**AA** = Normal Control  **AS** = Sickle cell trait  
**SCD** = Sickle cell disease  **Hyd+** = hydroxyurea therapy present  
**Hyd-** = Hydroxyurea therapy absent

Table 2: Values of haematological and other parameters

<table>
<thead>
<tr>
<th>Haematological and other Parameters</th>
<th>AA</th>
<th>AS</th>
<th>SCD Hyd+</th>
<th>SCD Hyd-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (g%)</td>
<td>12.73 ± 2.18</td>
<td>11.8 ± 2.31</td>
<td>10.75 ± 1.70</td>
<td>7.32 ± 1.26</td>
</tr>
<tr>
<td>RBC count(X10 <strong>12/L</strong>)</td>
<td>4.21 ± 0.59</td>
<td>4.11 ± 0.60</td>
<td>3.75 ± 0.66</td>
<td>2.98 ± 0.75</td>
</tr>
<tr>
<td>TLC (/Cumm)</td>
<td>7.46 ± 2.60</td>
<td>7.16 ± 2.12</td>
<td>9.04 ± 3.67</td>
<td>11.09 ± 3.70</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>87.51 ± 9.70</td>
<td>79.22 ± 10.74</td>
<td>80.42 ± 8.36</td>
<td>78.30 ± 10.11</td>
</tr>
<tr>
<td>MCH (hg)</td>
<td>30.28 ± 4.29</td>
<td>27.66 ± 3.88</td>
<td>27.69 ± 2.83</td>
<td>26.31 ± 3.76</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>34.58 ± 1.84</td>
<td>34.98 ± 1.32</td>
<td>34.54 ± 1.33</td>
<td>33.65 ± 1.44</td>
</tr>
<tr>
<td>Total Bilirubin (mg%)</td>
<td>0.54 ± 0.22</td>
<td>1.01 ± 0.52</td>
<td>1.51 ± 0.88</td>
<td>2.40 ± 1.12</td>
</tr>
</tbody>
</table>
Reference


USEFULNESS OF LEVELS OF C - REACTIVE PROTEIN IN DIFFERENTIATING BETWEEN PULMONARY TUBERCULOSIS AND NON-TUBERCULAR BACTERIAL PNEUMONIA

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Abstract
Differential diagnosis between pulmonary tuberculosis (TB) and non-tubercular bacterial pneumonia is important as prompt isolation of pulmonary tuberculosis patients and initiation of treatment is necessary. The aim of the study was to explore means of discriminating between the two, based on levels of C-reactive protein (CRP). 100 patients presenting with signs and symptoms of pneumonia formed the study group and were compared with 25 age and sex matched healthy controls. Levels of CRP were estimated by standardized immunoturbidimetric methods on HITACHI 902. Sensitivity and specificity of a range of CRP levels was investigated and the cut off values that resulted in best discrimination were calculated using Youden’s index. 54 patients were diagnosed with tubercular pneumonia and 46 had non-tubercular bacterial pneumonia. The levels of CRP were significantly lower (p<0.001) in patients with TB as compared to non-tubercular bacterial pneumonia (median levels 4.25 mg/L and 23.9mg/L respectively). The best cutoff was found to be for CRP levels < 5mg/L, where the sensitivity for detecting TB was 78% and specificity was 56%. The positive predictive value (PPV) for CRP levels < 5mg/L was calculated to be 66.7% and negative predictive value (NPV) 65.7%. The high sensitivity and negative predictive value in differentiating between pulmonary tuberculosis and non-tubercular bacterial pneumonia suggests a supplementary role of CRP for the diagnostic exclusion of pulmonary TB from non-tubercular bacterial pneumonia in areas with high prevalence of the disease

Introduction
Mycobacterium tuberculosis is a frequent cause of pneumonia in areas with high burden of pulmonary tuberculosis\(^1\). Clinicians are frequently faced with the challenge of differentiating between pulmonary tuberculosis and pneumonia due to other bacterial pathogens. The varying clinical and radiographic presentation and a low sensitivity of AFB microscopy make it even more difficult to distinguish between the two\(^2\). Differential diagnosis between the two is important as prompt isolation of pulmonary tuberculosis patients and initiation of treatment is necessary. Serum levels of CRP, an acute phase reactant protein synthesized by the liver following stimulation by various cytokines including tumor necrosis factor-\(\alpha\) and interleukin-6 (IL-6), markedly increase within hours after infection or inflammation. Since pneumonia is an inflammation of the lung, C-reactive protein (CRP), could be used as a marker of inflammation to detect the extent of injury\(^3\). Numerous studies have demonstrated increased levels of CRP in patients with pulmonary tuberculosis and non-tubercular bacterial pneumonia, but it’s utility to discriminate between the two has yet not been fully evaluated in our part of the world\(^4,5\).

Being an inexpensive and readily available test, we explored the usefulness of CRP levels as a useful adjunctive test in the early differentiation of pulmonary tuberculosis from non-tubercular bacterial pneumonia.
Materials and Methods
The study was conducted in the Department of Biochemistry & the Department of Pulmonary Medicine, Government Medical College & Hospital, Chandigarh, India. The study was planned as a descriptive cross-sectional and observational study comprising of 100 patients with signs and symptoms of pulmonary tuberculosis or bacterial pneumonia, attending the OPD of the Department of Pulmonary Medicine. Patients with previously diagnosed or on treatment of pulmonary tuberculosis or bacterial pneumonia and patients with any other systemic / infective illness were excluded from the study.

Clearance from the Institutional ethical and research committee was obtained and informed consent was taken from all participants of the study. Clinical data collected from the patients included vital signs, symptoms and Chest X Ray features. Lab measurements were conducted on the day of the enrolment. Levels of CRP were estimated in blood samples by standardized immunoturbidimetric methods on HITACHI 902 Autoanalyzer.

The diagnosis of TB and Pneumonia was done by the clinician. Patients were considered to have pulmonary TB when M. tuberculosis was cultured from the sputum or lavage fluid combined with a lung parenchymal lesion. Bacterial pneumonia was diagnosed when the patient had infiltrate on chest X-ray, which resolved completely with antibiotic treatment and culture of sputum was negative for M. tuberculosis.

The median levels of CRP in the study groups were statistically compared using Mann Whitney test. Sensitivity and Specificity of a range of CRP levels was investigated and the cutoff values that resulted in best discrimination were calculated using Youden’s index. The optimal cut-off levels were further investigated using receiver operating characteristic (ROC) analysis.

Results
The study group comprised of 100 patients presenting with the signs and symptoms of pneumonia. The demographic characteristics of the subjects of the study are shown in table 1. 54% of the cases were diagnosed with pulmonary tuberculosis and 46% had non-tubercular bacterial pneumonia. The patients in the two subgroups were age and sex matched. Incidence of cough, sputum and fever were found to be significantly more (p<0.001) in patients with non-tubercular pneumonia, while weight loss was found to be significantly more (p<0.001) in patients with pulmonary tuberculosis.

Levels of CRP were found to be significantly more (p<0.001) in the patients of non-tubercular bacterial pneumonia when compared to the patients of pulmonary tuberculosis. The best cut-off level for differentiating between pulmonary tuberculosis and non-tubercular bacterial pneumonia was found to be a CRP level of <5 mg/L with the highest Youden’s Index of 0.53, and a sensitivity of detecting pulmonary tuberculosis of 77% and specificity of 76% as shown in Table 2. CRP levels of <5 mg/L were found to have a positive predictive value of 79.25% (95% CI: 65.89%-89.14%) and a negative predictive value of 74.47% (95% CI: 59.65%-86.04%) for diagnosis of pulmonary tuberculosis in the patients of the study group.

Fig. 1 shows the Receiver Operating Characteristic curve for levels of CRP. Area under ROC for levels of CRP <5 mg/L was found to be 0.74, suggestive of the usefulness of these levels in discriminating between pulmonary tuberculosis and non-tubercular bacterial pneumonia.

Discussion
India has the highest burden of TB in the world, an estimated 2 million cases annually, and accounting for approximately one fifth of the global incidence. It is estimated that about 40% of the Indian population is infected with TB.
bacteria, the vast majority of whom have latent rather than active TB disease. It is also estimated by the World Health Organization (WHO) that 300,000 people die from TB each year in India\(^7\).

Pulmonary tuberculosis is a granulomatous infectious disease of lung caused by Mycobacterium tuberculosis. There are varied presentations of pulmonary tuberculosis, pneumonia being the most common\(^8\).

Non-tubercular bacterial pneumonia is an acute inflammation of lung parenchyma most commonly caused by Streptococcus pneumoniae\(^9\). It is the third leading cause of death worldwide with a high rate of hospitalization; complication and mortality\(^10\). Almost 20% of the hospitalized patients need intensive care admission and 10% of progress to non-resolving pneumonia\(^8,11\).

A rapid diagnosis and appropriate antibiotic treatment are therefore, essential to reduce the morbidity and mortality of Pneumonia. In areas like ours with a high burden of Tuberculosis, the differential diagnosis of pulmonary tuberculosis from non-tubercular pneumonia becomes difficult owing to the similar clinical presentations. Sputum culture for Mycobacterium tuberculosis is the gold standard for diagnosis and sputum Z-N staining is most commonly used. The AFB smear test lacks specificity\(^12\). These sputum negative patients can be differentiated from non-tubercular pneumonia by other modality of investigation (sputum culture for Mycobacterium, Bronchial lavage or Histopathological examination) which are time taking, costly and require specialist. Therefore an adjunct diagnostic test that can differentiate between pulmonary tuberculosis and non-tubercular bacterial pneumonia at an early stage of the disease will be of great clinical importance in terms of isolating patients with pulmonary tuberculosis and administering appropriate anti-tubercular medication or antibiotic treatment.

CRP is an acute phase reactant, synthesized by hepatocytes under the influence of interleukin-1 arising at sites of infection, inflammation and trauma\(^3\). The ability of serum CRP levels to identify etiology of pneumonia and predict prognosis has been investigated\(^4,5\). The utility of levels of CRP as a marker of bacterial infection in the lower respiratory tract has also been studied in several populations\(^13\). In a study conducted by Choi M et al, median CRP levels were found to be lower in patients with tuberculosis as compared to patients with non-tubercular pneumonia\(^14\). In a similar study conducted by Young Ae Kang et al, the role of levels of CRP in differentiating between pulmonary tuberculosis and pneumonia was emphasized\(^15\).

The results of our study also suggest the usefulness of serum levels of CRP in distinguishing between pulmonary tuberculosis and non-tubercular bacterial pneumonia. We found significant low levels of CRP in patients with tuberculosis as compared to those with non-tubercular bacterial pneumonia. We report the best cut-off for distinguishing between the two as serum CRP levels of <5 mg/L suggestive of pulmonary tuberculosis, with a high sensitivity and negative predictive value.

Thus, in conclusion, we suggest that serum levels of CRP might have an important role in the diagnostic algorithm of pneumonia as rapid, inexpensive and non-invasive test for the exclusion of pulmonary tuberculosis from non-tubercular bacterial pneumonia in areas with high prevalence of the disease.

**Acknowledgement**

We acknowledge the financial support extended by The Department of Science & Technology, Chandigarh Administration, for carrying out this work.
Table 1: Demographic profile of subjects of the study group

<table>
<thead>
<tr>
<th>Types of Pneumonia</th>
<th>Non-tubercular bacterial pneumonia</th>
<th>Pulmonary tuberculosis</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>40.57±16.35 years</td>
<td>38.40±16.32 years</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>Sex</td>
<td>Males 66.6%</td>
<td>60.3%</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td></td>
<td>Females 33.4%</td>
<td>39.7%</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>Incidence of symptoms</td>
<td>Sputum 93%</td>
<td>76%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Cough 95.6%</td>
<td>71.7%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Fever 91.10%</td>
<td>65.2%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Weight loss 27.10%</td>
<td>53.50%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Median Levels of CRP (mg/L)</td>
<td>23.90</td>
<td>4.25</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 2: Number of patients according to level of CRP, using a range of cutoffs, sensitivities and specificities

<table>
<thead>
<tr>
<th>CRP levels</th>
<th>No. of patients</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Youden’s Index</th>
<th>1-specificity</th>
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<tbody>
<tr>
<td></td>
<td>Tubercular Pneumonia</td>
<td>Non-tubercular Pneumonia</td>
<td></td>
<td></td>
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<tr>
<td>&lt; 2 mg/L</td>
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<td>7</td>
<td>0.33</td>
<td>0.84</td>
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<tr>
<td>&lt;5 mg/L</td>
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<td>11</td>
<td>0.77</td>
<td>0.76</td>
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<tr>
<td>&lt;10 mg/L</td>
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<td>19</td>
<td>0.88</td>
<td>0.58</td>
<td>0.46</td>
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<td>&lt;15 mg/L</td>
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<td>31</td>
<td>0.96</td>
<td>0.32</td>
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Fig. 1: Receiver Operating Characteristic curve for levels of CRP in patients of the study group

Reference

Introduction

Vitamin-D is predominantly known for its importance in calcium metabolism and homeostasis. Emphasis has now been shifting from the involvement of vitamin-D in bone health to its non-skeletal concern. The expression of Vitamin D receptors (VDR) is noted in most tissues and cells of the human body including liver, pancreas, and several immune cells including monocytes, macrophages, T lymphocytes, B lymphocytes, natural killer (NK) cells, and dendritic cells (DC), with expression most abundant on the epithelial cells of the gastrointestinal tract. The pleotropic functions of vitamin D including anti-inflammatory, anti-apoptotic, anti-fibrotic roles has been gaining influence in recent years[1]. The production of vitamin-D on exposure to sunlight depends on incident angle of the sun and latitude, season, time of sun exposure and duration, skin pigmentation, aging and sunscreen usage. Recent studies have shown increased incidence of vitamin-D deficiency throughout the world and its association with various co-morbid conditions[2,3].

Contradictory to the belief that vitamin-D deficiency must be a rare occurrence in tropical country like India receiving sunlight throughout the year, studies have shown the prevalence of vitamin-D deficiency in our country upto an extent of 90%[4]. However, data to reflect on the magnitude of vitamin-D deficiency and its association with various co-morbidities is very scarce. So this study is carried out with the objective of assessing vitamin-D levels in a hospital patient population and its association with co-morbid illnesses. Estimated Vitamin-D levels were obtained from the medical records who were admitted to a tertiary care hospital during the period of August 2012-March 2014. Details of the patients including age, gender, diagnosis and associated co-morbidities were taken from the medical records. The study included 450 in-patients out of which 231 were males and 219 females. Our study showed that the prevalence of vitamin-D deficiency was 83% which was nearly equal in males and females. Further analysis showed that the most common morbidities associated with vitamin-D deficiency was type 2 diabetes mellitus and systemic hypertension. Nearly 34.6% of vitamin-D deficient subjects had chronic liver disease and neurological disturbances each. The other common co-morbidities associated were cancer, thyroid disorders, tuberculosis and autoimmune diseases.

Epidemic of vitamin-D deficiency in India is likely to significantly contribute to the enormous burden on the healthcare system. Adequate supplementation of vitamin-D to the population could have a major impact in the prevention of vitamin-D deficiency and thereby reducing the trouble caused by the most common co-morbidities associated with its deficiency.
vitamin-D deficiency and to create an insight into the prevalence of vitamin-D deficiency in hospitalised patients and its association with various co-morbid conditions in our country is very scarce. So this study is carried out with the objective of assessing vitamin-D levels in a tertiary care hospital and associated co-morbid conditions.

Materials and Methods
Estimated Vitamin-D levels were obtained from the medical records who were admitted to a tertiary care hospital during the period of August 2012-March 2014, after the clearance from Institutional Human Ethics Committee. Details of the patients including age, gender, diagnosis and associated co-morbidities were taken from the medical records. Serum 25-hydroxy vitamin-D was measured in Roche e411 auto-analyzer using dedicated kits by Electrochemiluminescence method. Normal serum reference interval was 30-70ng/mL. Vitamin-D levels of 20-<30ng/mL was classified as vitamin-D insufficiency and levels <20ng/mL were classified as vitamin-D deficiency (VDD). VDD was further classified based on Lips classification as mild (10-<20ng/mL), moderate (5<10ng/mL) and severe (<5ng/mL)(5,6).

Results
A Vitamin-D levels of 450 patients attending to a tertiary care hospital were included and analysed in the study after obtaining clearance from Institutional Human Ethics Committee. Mean age of the study population was 51.50±17.48 years. The study group included 231 males and 219 females. The age-wise distribution of the study population is depicted in Fig 1. The prevalence of vitamin-D deficiency in males and females are illustrated in the graph in Fig 2. Out of the 450, 389 (86.4%) had low vitamin-D levels.

Discussion
In the present study, it was revealed that out of 450 in-patients in whom vitamin-D levels were estimated, 389 patients (86.4%) had low vitamin-D levels (<30ng/mL). Further the prevalence of low vitamin-D levels were nearly equal in both males (84%) and females (89%). The most common age group in which vitamin-D levels were insufficient was 41-60 years which is consistent with previous studies which reported older adults are at increased risk of developing low vitamin-D levels (<30ng/mL). This is partly because as age advances, skin cannot synthesize vitamin-D efficiently. Also they are likely to spend more time indoors and have inadequate intake of the vitamin(7).

The most common co-morbid condition associated with vitamin-D deficiency in our study was type 2 diabetes mellitus. In our study, it was found that 47% of patients with severe vitamin-D deficiency had diabetes mellitus. Lee et al. found that 89% of their study population of diabetic patients suffered from vitamin-D deficiency and just 9 out of 300 persons had sufficient vitamin-D concentration(8). This might be due to the direct role of vitamin-D in the activation of pancreatic beta-cell or indirect role by regulation of calcium homeostasis.

The next common illness associated with vitamin-D deficiency was found to be arterial hypertension. In our study about 30% of the patients with decreased vitamin-D levels had associated systemic hypertension. This supports the antihypertensive and vascular protective effects of vitamin-D, such as suppression of the renin-angiotensin-aldosterone system and anti-atherosclerotic properties including improvements of endothelial function(9).

The co-morbid condition associated with vitamin-D deficiency in about 34.6% of subjects with low vitamin-D levels had chronic liver disease. Impaired conversion of vitamin-D to its 25-hydroxylated form in the liver is the major mechanism for the resulting vitamin-D insufficiency in these patients. Photo conversion in skin is normal in these patients(10). Our findings were consistent with the findings of Fisher et al.
which reported the prevalence of vitamin-D deficiency high in liver disease patients (86.3%)\(^{(11)}\). The other common morbidities associated with vitamin-D inadequacy in decreasing order of frequency were neurological disturbances, thyroid dysfunction, malignancy, respiratory diseases and autoimmune diseases. Nearly an equal number of patients had neurological disturbances as liver disease. Vitamin-D has been found to function as a modulator in brain development and neuro-protectant. It also exhibits an association with the regulation of nerve growth factor synthesis which is responsible for the growth and survival of neurons\(^{(12)}\).

About 21.3% of vitamin-D deficient individuals had thyroid dysfunction. Extensive studies in the association of vitamin-D inadequacy and thyroid dysfunction are scarce. Amal Mackawy and his co-workers\(^{(13)}\) studied the effect of vitamin-D deficiency on thyroid gland in experimental study and reported that a lack of vitamin-D contributed to the possibility of low thyroid hormones.

In our study, 13 out of 291 vitamin-D deficient individuals had tuberculosis. This was consistent with recent meta-analysis which showed that Vitamin-D deficiency was associated with higher risk of active tuberculosis\(^{(14)}\). Vitamin-D was established to have a protective role in the treatment of tuberculosis about 6 decades ago\(^{(15)}\), but the idea was unobserved. Vitamin-D deficiency has been found to develop as a pandemic in recent years. It has also been associated with several co-morbid conditions which contribute the huge economic burden on our country. Vitamin-D estimation should be included in routine laboratory analysis and vitamin-D supplements provided to reduce the risks associated with vitamin-D deficiency and its associated co-morbidities.

**Conclusion**

Vitamin-D deficiency is likely to play an important role in the very high prevalence of rickets, osteoporosis, cardiovascular diseases, diabetes, cancer and infections such as tuberculosis in India. Owing to its multifarious implications on health, the epidemic of vitamin-D deficiency in India is likely to significantly contribute to the enormous burden on the healthcare system of India. Most of the widely consumed food items such as dairy products are rarely fortified with vitamin-D. Fortification of staple foods with vitamin-D is the most viable population based strategy to achieve vitamin-D sufficiency. Vitamin-D supplements are available at affordable prices, so that proper supplementation of the vitamin to the population could have a major impact in the prevention of vitamin-D deficiency and thereby reducing the trouble caused by the most common co-morbidities associated with its deficiency.
Figure 1: Age-wise distribution of study population

Figure 2: Vitamin-D status in males and females
### Table 1: Vitamin D deficiency associated co-morbidities in percentage

<table>
<thead>
<tr>
<th>Disease associated</th>
<th>Severe deficiency</th>
<th>Moderate deficiency</th>
<th>Mild deficiency</th>
<th>Vitamin-D insufficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males Total 35</td>
<td>Females Total 37</td>
<td>Males Total 46</td>
<td>Females Total 49</td>
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<tr>
<td>Liver diseases</td>
<td>37</td>
<td>8</td>
<td>30</td>
<td>4</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>49</td>
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<td>33</td>
<td>10</td>
</tr>
<tr>
<td>Hypertension</td>
<td>23</td>
<td>41</td>
<td>33</td>
<td>10</td>
</tr>
<tr>
<td>Cancer</td>
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<td>2</td>
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<td>Respiratory diseases</td>
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<td>5</td>
<td>7</td>
<td>0</td>
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<td>Autoimmune diseases</td>
<td>11</td>
<td>5</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Neurology</td>
<td>14</td>
<td>27</td>
<td>13</td>
<td>31</td>
</tr>
<tr>
<td>Thyroid</td>
<td>0</td>
<td>22</td>
<td>4</td>
<td>8</td>
</tr>
</tbody>
</table>

### Reference

7. Institute of Medicine, Food and Nutrition Board. Dietary Reference Intakes for Calcium and Vitamin-D. Washington, DC: National Academy Press, 2010
CLINICOPATHOLOGICAL PROFILE OF NONALCOHOLIC FATTY LIVER DISEASE (NAFLD) AND ITS ASSOCIATION WITH METABOLIC SYNDROME

M. M. Chatterjee, Quazi. S. Haque, F. Jamal

Abstract

All patients attending the health checkup had their blood pressure, height and weight, waist circumference measurements, blood sugars, lipid levels and ultrasound abdomen done. The prevalence of NAFLD among these subjects was determined and the presence of risk factors for metabolic syndrome in each individual was analyzed. A relationship between NAFLD and metabolic syndrome was then established. Result of the 1003 people 225 (22.6%) had NAFLD with higher prevalence among males 164/565 (29%) than among females 61/438 (13.9%). In the NAFLD group normal body mass index (BMI) was present in only 49/225 (20%) of the subjects while 119/225 (52.8%) were overweight and 56/225 (24.8%) were obese. Though liver enzymes were normal the mean AST among cases was 37.41 + 14.50 and 33.93 + 14.15 among controls and the mean ALT was 38.74 + 17.96 among cases and 31.62 + 13.49 among controls. Prevalence of metabolic syndrome was 106/225 (47%) among cases and 179/778 (23%) among controls. A diagnosis of fatty liver on ultrasound in an asymptomatic person should be taken as an indicator of metabolic syndrome and its progression to cardiovascular disease.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most common cause of liver dysfunction worldwide. NAFLD may progress to non-alcoholic steatohepatitis (NASH) and in turn Cirrhosis[1]. All stages are associated with accumulation of fat in the liver cells[2]. NAFLD was initially believed to be a benign condition. Recent studies have shown it to be associated with obesity, type II diabetes mellitus, dyslipidemia and hypertension with insulin resistance being a common factor. These conditions cluster to form the metabolic syndrome, which causes high risk for cardiovascular disease[3]. The diagnosis of NAFLD requires a combination of invasive and non-invasive tests. Mild to moderately elevated serum levels of aspartate amino transferase (AST) and alanine aminotransferase (ALT) or both are the most common findings[4]. However some studies suggested that use of liver enzymes as a marker of NAFLD underestimate its prevalence[5]. Ultrasound has a sensitivity of 89% and a specificity of 93% in detecting steatosis, sensitivity and specificity of 77% and 89% in detecting increased fibrosis[6]. This study was conducted with the aim of determining the prevalence of NAFLD in the apparently healthy population and establishing a relationship between NAFLD and metabolic syndrome.
Materials and Methods
All the patients of age group 20-60 years attending OPD for routine health checkup formed the control group. They were subjected to their height, weight, waist circumference measurements, blood pressure, BMI, Liver function test, lipid profile, fasting blood glucose (FBS), post prandial blood sugar (PPBS) and ultrasound examination of abdomen. NAFLD was defined as fatty liver not resulting from excessive alcohol consumption on (>20grams/day) drugs, toxins infection diseases or any other identifiable exogenous cases(7). This was an ultrasound based study. USG findings revealed that fatty liver was diagnosed as diffuse increase in parenchymal ecogenicity with progressive loss of clarity of portal veins and increased attenuation of sound by the liver(8). Those with USG evidence of fatty liver along with levels of AST > 55u/dl and ALT > 70 u/dl had their blood further investigated for thyroid stimulating hormones, hepatitis B virus, hepatitis C virus, serum ceruloplasmin, alpha-1 antitrypsin levels and serum transferring levels to rule out other causes of liver disease. The diagnosis of NAFLD was made only after excluding them. Normal weight has been defined as BMI from 20-25, over weight from 25-30 and obesity from 30-35. Metabolic syndrome was identified by finding any three of the five risk factor(9). Those with normal ultrasound formed the control group. All the observation and data were analyzed in the statistical package social sciences (SPSS). The level of significance was set as P<0.05.

Results
The health checkup was attended by 1026 people of which 23 had to be excluded due to significant alcohol consumption. Hence our study group comprises of 1003 people which had 438 females and 565 males. Of the 1003 people 225 people had USG evidence of fatty liver. Thus the prevalence of NAFLD was 225/1003 (22.6%). It was present among 61/438 (13.9) females and 164/565 (29%) males. Of the 225 people with NAFLD only 1 person (0.4%) was underweight with a BMI less than 20. Normal BMI was observed in normal and overweight respectively 49(21.7%) 119(52.8%), while 56(24.8%) of them had frank obesity. Among those with sonographic evidence of fatty liver, only 12 subjects had elevated transaminases yet one had values correspond to the definition of NASH which meant elevated liver enzymes by 1.5 to 5 times. Although most of the subjects in present study (Table 1) had liver enzymes with in the normal range yet the mean values were higher with AST being 37.41+14.50 in cases and 33.93+17.96 and 31.62+13.49 among cases and controls respectively. Hypertriglyceridemia was present in the NAFLD group with mean of 170.02+88.90 mg% and among controls it was 132.56+69.77 mg%. Levels of low density lipoproteins (LDL) were 109.18+29.72 mg% and 107.08+32.18 mg% respectively for cases and controls. The total cholesterol levels were 187.92+36.72 mg% for cases and controls. The total cholesterol levels were 187.92+36.32 mg% for cases and 181.81+37.33 mg% for controls. The low HDL level was low for cases with mean being 46.61+9.48 mg% and 49.60+12.74 mg% for controls. The FBS was elevated with cases having 124.62+45.83 mg% and controls with 109.89+37.80 mg%. Post prandial sugars were higher in cases 156.93+78.33 mg% and among controls it was 129.59+63.70 mg%. The BMI was higher in NAFLD group, mean of 28.58+4.25 and among controls it was 25.67+5.05. Metabolic syndrome (Table 2) was present in 106/225(47%) of cases and 179/778(23%) of controls. Impaired blood glucose levels were seen in 163/225(72.4%) of cases and 438/778 (56.3%) of controls. Hypertension was prevalent
in 64/225 (28.4%) of NAFLD group as against 147/778 (18.9%) of controls. Elevated triglycerides were present in 98/225 (43.6%) of cases and 213/778 (27.4%) of controls and low HDL levels were seen in 66/225 (29.3%) of cases and 247/778 (31.7%) of controls. Abdominal obesity was present in 106/225 (47.1%) of cases and 303/778 (38.9%) of controls. Other than HDL, all had significant P value at P<0.05. Not even a single risk factor of metabolic syndrome (Table 3) was present in 10/225 (0.4%) of cases and 124/778 (15.9%) of controls, while one risk factor was prevalent in 37/225 (16.4%) of cases and 258/778 (33.2%) of controls. The presence of two risk factors were similar in both groups at 63/225 (28%) among cases and 206/778 (26.5%) among controls. Three risk factor were present in 66/225 (29.3%) of NAFLD group and 129/778 (16.6%) of controls. Four risk factors were noted in 37/225 (16.4%) and 46/778 (5.9%) of cases and controls respectively. All the component of metabolic syndrome were present in 9/225 (4%) of cases and 6/778 (0.8%) of controls.

Discussion
NAFLD has been reported in 10% to 24% of the general population in various countries\textsuperscript{[10]}. An USG based study conducted in India has shown a prevalence of 24.5%\textsuperscript{[11]}. The present study showed a prevalence of 22.6% with a higher prevalence among male than among females. The results of the present study were almost similar to that study. Marchesani et al. showed that 80% of patients with NAFLD were Obese\textsuperscript{[12]}. Our study had only 21% of the population with normal BMI while the remaining 79% were either overweight or obese. Although majority of the cases had normal liver enzymes the mean values of the enzymes were higher in the NAFLD group as compared to the controls.

Goland et al. observed that patients with NAFLD had a significantly higher BMI of 31.14 vs 26.4, higher blood glucose level of 100.6 vs 83.3 and triglyceride values of 200 vs 126 respectively\textsuperscript{[13]}. Lee et al. showed higher anthropometrics value among controls. The ALT and AST levels were higher with an increase in total cholesterol, triglycerides, atherogenic index, FBS, systolic and diastolic blood pressure and a decrease in HDL cholesterol in the USG diagnosed patients as against controls. There was no significant difference in the LDL cholesterol values among both the groups\textsuperscript{[14]}. There is a strong association between NAFLD and metabolic syndrome with its prevalence being double in the NAFLD group as compared to the controls. The liver histology is closely associated with the number of risk factors\textsuperscript{[15]}. Our study showed the increased presence of number of risk factors of metabolic syndrome in those with ultrasonic evidence of fatty liver as compared to the controls NAFLD can rightly be called the hepatic component of metabolic syndrome. This was a fact finding study. Currently, this disease is a public health problem world wide, due to the high prevalence, high level of disability associated and high cost for the health system\textsuperscript{[16,17]}. Conclusion
We thus concluded that evidence of fatty liver should be taken seriously as a predictor of metabolic syndrome. It is atherogenic and predisposes to diabetes, hypertension, dyslipidimia and has a strong potential for coronary vascular disease. Prevention is better than cure. Hence a diagnosis of NAFLD on ultrasound in an asymptomatic patient should alert as of the preventable metabolic syndrome and its progression to coronary vascular disease making it necessary to take the same precautions we would take for any other predictors of metabolic syndrome.
Table 1: Comparison of lipid profile, glucose level and anthropometric values between the two groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean + SD NAFLD Group (N=225)</th>
<th>Control Group (N=225)</th>
<th>P Value</th>
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<tbody>
<tr>
<td>HDL (mg%)</td>
<td>46.61 + 9.48</td>
<td>49.60 + 12.74</td>
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<tr>
<td>LDL (mg%)</td>
<td>109.18 + 29.72</td>
<td>107.08 + 32.18</td>
<td>&lt;0.05</td>
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<tr>
<td>FBS (mg%)</td>
<td>124.62 + 45.83</td>
<td>109.89 + 37.80</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>PPBS (mg%)</td>
<td>156.93 + 78.83</td>
<td>126.59 + 63.70</td>
<td>-</td>
</tr>
<tr>
<td>Total Cholesterol (mg%)</td>
<td>187.92 + 36.32</td>
<td>181.81 + 37.33</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>TGL (mg%)</td>
<td>170.02 + 88.90</td>
<td>132.56 + 69.77</td>
<td>&lt;0.05</td>
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<tr>
<td>SGOT (μ/dl)</td>
<td>37.41 + 14.50</td>
<td>33.93 + 14.15</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>SGPT (μ/dl)</td>
<td>38.74 + 17.96</td>
<td>31.62 + 13.49</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>BMI (cm)</td>
<td>28.58 + 4.25</td>
<td>25.67 + 5.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>99.96 + 10.01</td>
<td>93.22 + 11.04</td>
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Table 2: Prevalence of different parameters between two study groups

<table>
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<tr>
<th>Total No. of Patients enrolled</th>
<th>NAFLD (N=225)</th>
<th>Control (N=778)</th>
<th>P Value</th>
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</thead>
<tbody>
<tr>
<td>Prevalence of metabolic syndrome</td>
<td>106(47.1%)</td>
<td>179(23.0%)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Prevalence of impaired blood glucose (FBS&gt;100mg %)</td>
<td>163(72.4%)</td>
<td>438(56.3%)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Prevalence of hypertension (B.P.&gt;130/85)</td>
<td>64(28.4%)</td>
<td>147(18.9%)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Prevalence of elevated triglycerides (&gt;150mg/dl)</td>
<td>98(43.6%)</td>
<td>213(27.4%)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Prevalence of low HDL (&lt;40mg% in males &amp; &lt;50 mg% in females)</td>
<td>66(29.3%)</td>
<td>247(31.7%)</td>
<td></td>
</tr>
<tr>
<td>Prevalence of increased waist circumference (&gt;88cm. in females &amp; &gt;102cm. in males)</td>
<td>106(47.1%)</td>
<td>303(38.9%)</td>
<td>&lt;0.05</td>
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Table 3: Distribution of risk factors among study groups

<table>
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<th>Risk Factor</th>
<th>NAFLD Group (N=225)</th>
<th>Control Group (N=778)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>No Risk Factor</td>
<td>10</td>
<td>0.04%</td>
<td>124</td>
</tr>
<tr>
<td>1 Risk Factor</td>
<td>37</td>
<td>16.4%</td>
<td>258</td>
</tr>
<tr>
<td>2 Risk Factor</td>
<td>63</td>
<td>28.0%</td>
<td>206</td>
</tr>
<tr>
<td>3 Risk Factor</td>
<td>66</td>
<td>29.3%</td>
<td>129</td>
</tr>
<tr>
<td>4 Risk Factor</td>
<td>37</td>
<td>16.4%</td>
<td>46</td>
</tr>
<tr>
<td>5 Risk Factor</td>
<td>09</td>
<td>04.0%</td>
<td>06</td>
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Reference

1. American Cancer Society. Cancer Facts and Figure 2013 Atlanta.
4. Bailey & Love’s. Short Practice of Surgery; 23rd Edn: 1227-1229
15. Dietel M, Arps H, Klapdor R, Muller- Hagen S, Sieck


INTRODUCTION

Polycystic Ovarian Syndrome (PCOS) is sometimes referred to as Sclerocystic Ovarian Disease, Stein-Leventhal Syndrome and Polycystic Ovarian Disease (PCOD). PCOS is a complex, heterogeneous, polygenic endocrine disorder in women of reproductive age and is considered as a multifactorial reproductive, cosmetic and metabolic problem. The etiology of PCOS is not well understood and its pathophysiological and molecular basis is still a puzzle. PCOS is likely to be the result of a number of both genetic and environmental factors. Some of the contributing factors to PCOS also include a low level of chronic inflammation in the body and fetal exposure to male hormones. However, androgen excess and insulin resistance leading to hyperinsulinemia are considered to be the basic defects in PCOS that was described way back in 1921 by Archard & Theirs as “diabetes of bearded women”\(^1\). The world wide prevalence of PCOS syndrome is 6-10% and in its “classical” form may affect 5-7% of women\(^2\). PCOS is quite common in Asian population. A high prevalence of PCOS up to 35% is reported for the Indian women where the incidence and prevalence of PCOS in overweight and obese women is greater than 20\(^3\). Women with PCOS are at a higher risk for a number of illnesses, including high blood pressure, diabetes, heart disease and other cardiovascular problems and cancer of the uterus, ovary and breast\(^4\). PCOS also presents with a variety of biochemical abnormalities\(^5\). The most consistent abnormality is hypersecretion of androgens.

GENETIC POLYMORPHISM IN CYP17A GENE IN PATIENTS WITH POLYCYSTIC OVARIAN SYNDROME (PCOS) IN A SOUTH-INDIAN POPULATION

Arindam Basu\(^1\), Arghya Sur\(^2\), Hemontika Chakraborty\(^1\), Prabha Adhikari\(^3\)

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\(^3\)Department of Medicine, Kasturba Medical College Hospital, Attavar, Mangalore, Karnataka- 575001, India

Abstract

Our study was likely to explore the association with single nucleotide polymorphism in cyp 17a gene in patient with PCOS. The study was conducted in Kasturba Medical College Hospital, Mangalore & Kerala Medical College & Hospital, Mangode, Cherpulassery, Palakkad Dist, Kerala. A total of 103 female patients with a clinical diagnosis of PCOS attending KMC, hospital, Mangalore, Kerala Medical College & Hospital, Mangode, Cherpulassery, Palakkad Dist, Kerala, medicine outpatient department were included in the study after obtaining informed consent. Biochemical assays of hormones, lipid profile, GTT as well as study of genotype and allelic frequencies were done in the hospital central biochemistry laboratory. In the radiology department, USG of ovary for diagnosis of PCOS was done. Other clinical diagnosis of PCOS were done in Medicine department. In our study cyp17a gene is not found to be associated with PCOS in South Indian population.
Because of the high degree of heterogeneity of PCOS, it is suggested best to consider PCOS as increased androgens clinically (acne, excessive hair on face, abdomen, or thinning of scalp hair) or in the blood (total or free testosterone, DHEAS), with oligo-ovulation (cycles greater than every 35 days, low mid-luteal progesterone, monophasic basal body temperature.

Studies on etiology of PCOS has yielded some positive results but the controversy on the mode of inheritance (e.g. autosomal dominance, modified autosomal dominance, X-linked, multifactorial) still persists. Thus, there is a great need to identify the potential candidate genes that may have a modest effect individually and in groups in PCOS. Three general genetic models have been proposed namely, Single gene Mendelian model which predicts that there is single gene defect inherited in a recessive or dominant pattern and that woman who inherit this defect develop clinically evident PCOS. Multifactorial model where PCOS is considered as a multifactorial genetic disorder and women carrying this defect through inheritance or environmental factors will have increased risk of clinical PCOS. Variable expression single gene model where there is a combination of the above two models. It follows that a single gene defect is present but its expression is modified by environmental factors. Therefore, a woman who is genetically predisposed but not exposed to environmental factors may develop only subclinical forms of PCOS and not the full disorder. This theory explains the heterogeneous nature of the disorder.

Therefore, it can be said that what started initially as a gynaecological curiosity, over the years has become a subject of multisystem endocrinopathy known as PCOS. Considering certain lacunae in the area of genetics of PCOS as pointed out earlier, we propose to study the family history of PCOS subjects, not only for PCOS but also for its related conditions. This would help us to determine the pattern of inheritance of PCOS and related clinical presentations.

Aims
To identify the frequency of the genetic polymorphisms in cyp17a gene in patients with PCOS.

Materials and Methods
Study setting, design, sampling and period of the study
Out-patients and in-patients of Kasturba Medical College Hospital, Attavar, Mangalore, as well as Out-patients and in-patients of Kerala Medical College & Hospital, Mangode, Cherpulassery, Palakkad Dist Kerala diagnosed with polycystic ovarian syndrome were included into the study. The study was a descriptive, cross sectional type. Convenient sampling of subjects was done for the study. The project began in December, 2008 and ended in December, 2011 at Kasturba Medical College Hospital, Attavar, Mangalore. Further it has been repeated at Kerala Medical College & Hospital, Cherpulassery, Palakkad, Kerala, from December, 2013 to September, 2014. Almost same results were obtained.

Ethical approval
Institute's Research ethical committee approval was obtained for the study. After obtaining informed consent from each participant, hundred and three (103) patients with a clinical diagnosis of PCOS (where large family trees was known) were included in the study. All patients received a long, careful and simple explanation of the purposes of the study and its pathophysiological basis.

Criteria for the definition of PCOS:
The diagnosis of PCOS was made according to the ESHRE/ASRM criteria for the PCOS diagnosis (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004)\(^6\) based on the presence of two of the three following criteria: oligo- and/or anovulation (menstrual dysfunction), clinical and/or biochemical signs of hyperandrogenism and polycystic ovaries (PCO) at ultrasonogram\(^7\).

Menstrual dysfunction was considered when the
women had oligomenorrhea, defined by six or fewer cycles per year, each cycle with a length of more than 35 days, and/or when the patient had not had any menstrual bleeding for 3 consecutive months during the last year. Clinical hyperandrogenism was defined by the presence of hirsutism, represented by a hirsutism score of 8 or more. Hyperandrogenism could be clinical (hirsutism, alopecia and/or acne) or subclinical, with only an increase in serum testosterone and/or dehydroepiandrosterone sulfate\(^8\). Polycystic ovaries were diagnosed by pelvic sonography according to the Rotterdam conference criteria.

**Patients and Participants**

103 female patients with a clinical diagnosis of PCOS and their 291 first and second-degree relatives were included in the study after obtaining informed consent.

**Inclusion criteria for patients**

1. Post pubertal females aged up to 35 years.
2. Irregular periods (Must have six or fewer menses /year)
3. Have clinical or laboratory evidence of hyperandrogenism (hirsutism or elevated testosterone) and PCO on ultrasound, more than 9 follicles; [Rotterdam criteria].
4. Who have signed informed consent

**Exclusion criteria for patients**

1. Age > 35 years.
2. Diabetes Mellitus > 5 years
3. Confirmed malignancy.

Total sample size: (103+291) = 394

103 female patients with a clinical diagnosis of PCOS by Rotterdam criteria attended KMC, hospital, Attavar; Mangalore as well as Kerala Medical College & Hospital, Mangode, Cherpulassery, Palakkad Dist medicine outpatient department or obstetrics and gynecology department and their first and second-degree relatives; were included in the study after obtaining informed consent.

All the subjects including patients and their family members were interviewed in detail and examined for anthropometry such as BMI, Hirsutism /excess hair, Acne, Baldism, Acanthosis nigricans, Skin tags, Buffalo humps, Moonface, Double chin.

1. Biochemical assay such as; Serum fasting insulin, Cortisol, Testosterone, Dehydroxyepiandrostenedione, LH, FSH, TSH were done in all cases, along with fasting lipid profile.
2. Blood pressure was measured for all, an oral 2 hr GTT was performed after 75 gm of glucose for all patients.

**Genetic analysis**

Study of genotype and allelic frequencies were done by means of PCR- RFLP. DNA was extracted from heparinised or EDTA blood.

Ethical clearance was obtained from Manipal University institutional Ethical Committee as well as KMCH, Mangode, Cherpulassery institutional Ethical Committee after which the studies were performed.

**Collection of blood samples**

The blood samples were collected from the patients with PCOS from coastal districts of Karnataka state and Kerala state. Patient’s history was collected and pedigree was drawn. Clinical documentation was undertaken with the help of a physician. DNA was extracted from both test and control samples following the standard phenol-chloroform method. PCOS patients were considered as test and normal individuals of same family were used as control.

Though we recruited 50 female individual devoid of all phenotypical feature of PCOS and having family history of diabetes.

**Results**

Genetic Polymorphism: When we screened 103 PCOS patients in CYP17A gene for -34T>C.
The allele of gene CYP17A that contain C instead of T is designated as A2 allele. The unmutated allele with T was designated as A1 allele of CYP17A gene. When we screened 103 patients with PCOS and their 291 first, and second degree relatives with 50 controls for the presence of polymorphic allele, No patients (0.0%) were heterozygous carrier of polymorphic A2 allele (genotype A1A2). Among 50 patients 0(0%) carried the (A2) allele in the heterozygous state (A1A2). We found no association between - 34T>C polymorphism and PCOS patients (Table 1).

Discussion
Familial clustering of PCOS has been consistently reported, suggesting that genetic factors play a role in the development of the syndrome. Sisters, brothers, fathers, mothers, daughters and now even sons of women with PCOS have been found to have a higher risk for exhibiting either hyperandrogenic or metabolic (hyperinsulinemic) traits of the disorder and thus PCOS has become a 'family affair'.

In the present study we examined the prevalence of a polymorphism of gene CYP17 promoter and found no association as A1A2=0.0 and A2A2=0.0 genotype frequencies. Echiburu et al (2008)(11) reported a frequency of 37% in PCOS subjects with A2 allele in Chilean population, when they examined 159 women with clinical and hormonal evidences of PCOS and they concluded the possibility of this gene defect in PCOS to be associated with increase in body weight, abdominal adiposity and metabolic components.

Diamati-Kandarakis et al (2009)(12) describe the same gene cyp17 polymorphism in Greek population with 50 PCOS subjects and reported 58% were heterozygous carriers of the polymorphic allele and 8% carried A2 allele in homozygosity, they concluded although this base pair substitution is not a primary genetic defect in PCOS, it may aggravate in clinical picture of hyperandrogenemia, particularly when homozygosity exist.

Gharani et al (1997)(13) also reported a non significant association with cyp17 gene and PCOS, when they examined 96 PCOS women in white population.

In another study Marszalek et al (2001)(14) illustrate a similar result in Poland population, while they genotyped 56 PCOS women and concluded the T>C polymorphism of cyp17 gene is not associated with steroid hormone synthesis in PCOS and it is not a primary genetic defect in this disease.

The high incidence of PCOS in first degree relatives of the affected members, in previous studies(15,16), suggests a dominant pattern of inheritance. This is based on the assumption that at least 50% of the siblings of the PCOS probands are affected with the disorder(15). Twin studies on PCOS revealed an incidence of 50% has suggested a complex pattern of polygenic inheritance(17). Other studies have reported that 50% hirsutism cases among the affected sisters of PCOS(18). They have shown that some characteristics of PCOS inherited differed in proportion; e.g. PCO 73%, hyperandrogenemia 87% and hyperinsulinemia 66%. Another report has shown 22% of PCOS in affected sisters of the proband(16).

However when we analyzed clinical conditions associated with PCOS, we found a very high association suggesting autosomal dominant transmission. Break up data of the family history data showed nearly 20% of the fathers of pco probands having diabetes mellitus, hypertension obesity/dyslipidemia each. Also a similar percentage of mothers of the pco probands had the above metabolic syndrome characters. Among the siblings of pco probands, nearly 10% of them had diabetes mellitus, hypertension, obesity/dyslipidemia each. Also a similar percentage of mothers of the pco probands had the above metabolic syndrome characters. Among the uncles, aunts and grandparents of our pco probands, the percentage of diabetes mellitus, hypertension, obesity/dyslipidemia was less than 15%. When any one of the metabolic syndrome character such as diabetes mellitus, hypertension
or dyslipidemia was considered, we found prevalence of 50% and 54% among the first degree and second degree relatives of our PCOS subjects respectively. Hence this can be concluded that Cyp17a gene is not associated with PCOS in South Indian population.

**Acknowledgement**
We acknowledge to all the physicians and nurses of OBG and General medicines departments of KMCH, Manipal as well as Manipal University for financial support and allowing us to use instruments for this study. We acknowledge Royal Medical Trust to pursue this research work in Kerala Medical College & Hospital, Mangode, Cherpuassery, Kerala.

**Table 1: Genotype frequencies in CYP17a1 genes in patients with PCOS, relatives and controls**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Polymorphism</th>
<th>Genotype frequency</th>
<th>Genotype frequency</th>
<th>Genotype frequency</th>
<th>Genotype (X^2)</th>
<th>OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP17A1</td>
<td>- 34T&gt;C</td>
<td>PCOS</td>
<td>Relatives</td>
<td>Controls</td>
<td>P value</td>
<td>OR</td>
</tr>
<tr>
<td>(RS-743572)</td>
<td></td>
<td>TT-.1</td>
<td>TT-.1</td>
<td>TT-.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>TC-.00</td>
<td>TC-.00</td>
<td>TC-.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CC-.00</td>
<td>CC-.00</td>
<td>CC-.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Reference**


ELEVATED ADVANCED OXIDATION PROTEIN PRODUCTS (AOPP) CORRELATES WITH HBA$_{1C}$ IN TYPE 2 DIABETES MELLITUS

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Abstract
Incidence & prevalence of type 2 DM is increasing in developing countries like India as also its chronic complications. Oxidative stress is believed to be a common pathogenic factor for these grave consequences. Advanced oxidation protein product (AOPP) is a marker of protein damage whereas total oxidant status (TOS) reflects the severity of oxidative stress. Different results were found by different authors about correlations between these markers and glycemic control. In this cross sectional study, 50 patients of clinically diagnosed type 2 DM & 28 age and sex matched controls were selected, according to inclusion and exclusion criteria. Disease population was further divided in two subgroups A & B, based on a median HbA1C level of 7.5%. (HbA1c was estimated by boronate affinity chromatography). We analysed the AOPP and TOS values in diabetes patients and compared them with controls. (They were estimated spectrophotometrically). We also tried to find out any correlation between the subgroups and oxidative parameters (AOPP and TOS). Significant increase in mean HbA1C, AOPP(p<0.0001) and increase in mean TOS level (p<0.086) were found in diabetes patients in comparison to controls. Significant correlations between HbA1C & AOPP was observed when HbA1C>7.5% (r=0.59p<0.001 y=74.71 + 9.26x). Hence AOPP estimation is very much beneficial in type 2 diabetes patients when HbA1C is >7.5% for prediction of chronic and grave complications of type 2 diabetes.

Introduction
The incidence and prevalence of type 2 diabetes mellitus (T2DM) is increasing in developing countries$^{(1)}$. With rapid development of therapy, the mortality from acute complications of diabetes has decreased, but mortality from chronic complications like diabetic nephropathy has increased$^{(2)}$. A currently favoured hypothesis is that oxidative stress, through a single unifying mechanism of superoxide production, is the common pathogenic factor leading to insulin resistance, β-cell dysfunction, impaired glucose tolerance (IGT) and ultimately to T2DM and its associated micro as well as macro vascular complications$^{(3,4)}$. Hyperglycaemia and oxidative stress have been implicated in the accelerated vascular damage associated with diabetes, which eventually manifests microvascular complications such as retinopathy, neuropathy, nephropathy and macrovascular disease e.g. peripheral arteriosclerosis, coronary disease, myocardial infarction and stroke. The vicious cycle mechanism between glycation and oxidation (‘glyco-oxidation’) has been proposed as one of the most important pathways. This leads to multiple biochemical sequels, which display a disturbance of oxidative–antioxidative balance, and creates glyco-oxidative molecular damage$^{(5,6)}$. The majority of the glyco-oxidation products brings about tissue degeneration, particularly in areas of blood vessels and thus take part in the origin of the diabetic vascular late complications e.g. nephropathy$^{(7,8)}$. Several studies suggested that, diabetes is associated with increased modification of proteins.
In addition to the formation and accumulation of advanced glycation end products (AGEs), a family of oxidized protein compounds termed advanced oxidation protein products (AOPPs), has emerged as a novel class of inflammatory mediators. AOPPs are the dityrosine containing and cross linked protein products (9,10), first described by Witko-Sarasat et al (1996) (11). These compounds are recognized as markers of oxidative damage to proteins, the intensity of OS and Inflammation (12). Studies have already suggested that AOPP plays a major role in the development of diabetic nephropathy, 8,10,13 as well as diabetic retinopathy (14). This structural and functional changes in diabetic nephropathy take place in the kidney during the early phases of diabetes, prior to microalbuminuria (15,16,17).

Human bodies are constantly protected against excessive oxidative stress by a complex set of enzymatic and non-enzymatic antioxidant systems (18). Low levels of ROS are indispensable in many biochemical processes (19); however, overproduction and/or inadequate removal of ROS can result in oxidative stress. Studies have also suggested that oxidative stress, in terms of Total Oxidant Status (TOS) is increased in patients with diabetic nephropathy as well as in diabetics without nephropathy and increase is related to the severity of diabetes (20).

Many authors submitted different opinions about altered values of AOPP and TOS in diabetes (6,13,20,21,22). However there is a few evidence of such studies at our region. Considering these facts we have tried to evaluate the values of those parameters in diabetics and to find out if any apparent correlations of them with glycemic control. Moreover, we tried to find out increased AOPP and TOS values can further worsen the hyperglycemia. This review will focus on identification of any oxidative stress factor to recognise and treat this devastating disease early in its progression and to postpone or even prevent the serious complications associated with it.

Materials and Methods
Study design
This hospital based, cross sectional, non interventional study was conducted during the period of 2010-12 in the Biochemistry department of NRS Medical College and Hospital, Kolkata, West Bengal, India.

Selection of the case group
The case group (Group-II) primarily included 50 (fifty) patients of clinically diagnosed T2DM (diagnosed from history and relevant biochemical tests), attending the outpatient department (OPD) and admitted in Medicine indoor ward of the institution on convenience basis. The patients were selected with the age group of 40-60 years following the inclusion and exclusion criteria. American Diabetic Association (ADA) targeted reasonable A1c goal as <7%, and less stringent A1c goal between 7-8% (23). Hence we have selected a median HbA1C level (7.5%), to subdivide the case group (n=50) into two groups (A and B), and to find out if any correlation persists between the subgroups and oxidative parameters. Group A comprises of 26 patients (HbA1C<7.5%), Group B comprises of 24 patients (HbA1C>7.5%).

Exclusion criteria for the case group
Hypertensive patients, T2DM patients with acute and chronic complications, severely ill, unconscious and disabled patients were not included in the study along with abnormal liver function tests. Patients with recent history of stroke, myocardial infarction or any disease which can cause oxidative stress were also excluded from the study.

Selection of the control group: 28 age and sex matched healthy control subjects (Group I) were also selected for the study. All control subjects were considered from more or less similar geographical area with similar socioeconomic status with no significant
difference in their food habit and drinking water quality.

**Exclusion criteria for control subjects**
Persons with chronic smoking habits, alcohol addiction or any drug addiction were excluded from the study.

**Ethical considerations**
Written consents (informed consents) were obtained from the participants (disease and control groups). The study protocol was approved by the ethics committee of N.R.S. Medical College.

**Sample Collection**
The amount of blood collected in absolute fasting condition with all aseptic precautions in 2 parts was 5 ml. The first part collected in ethylene diamine tetraacetic acid (EDTA) vial for estimation of glycated hemoglobin (HbA1c). The second part collected was allowed to clot and serum was separated for estimation of AOPP and TOS.

**Measurement of biochemical analytes**
HbA1c, the index of long term glycemic control, was determined with Micromat II (Biorad) instrument based on boronate affinity chromatography\(^{(24)}\).

**AOPP Assay**
Determination of AOPP (i.e. some oxidation products with characteristic absorbance) was based on spectrophotometric detection according to Witko-Sarsat et al. (1996) in our modification\(^{(25)}\). Concentration of AOPP is expressed in chloramine units (μmol/l).

**TOS Assay**
Serum TOS was measured using Erel’s TOS method\(^{(26)}\), which is based on the oxidation of ferrous ion to ferric ion in the presence of various oxidative species in acidic medium and the measurement of the ferric ion by xylenol orange. The results were expressed in μmol H\(_2\)O\(_2\) equivalent/l (μmol H\(_2\)O\(_2\) equiv./l).

**Data collection and processing for statistical analysis**
Statistical analysis was aimed
i) To assess the significance of difference between the mean values of serum AOPP and TOS between the Group I (controls) and Group II (cases).

ii) To assess the correlations between HbA1c and oxidative stress parameters (AOPP and TOS) in Group I (controls), Group A (HbA1c<7.5%) and Group B (HbA1c>7.5%).

**Statistical methods used**
Statistical analysis was done by one way and post hoc analysis of variance (ANOVA) with Bonferroni correction; P value was considered significant at the confidence level of 0.05. All statistical analysis were performed using SPSS software version 16.0 for windows.

**Results**
Table 1 shows that there is a significant increase in mean HbA1c and AOPP levels in diabetes in comparison to controls. The mean HbA1c level was found 8.3% in diabetics in comparison to 5.4% in controls (p<0.0001). The mean AOPP level was also observed as 139.6 μmol/L in T2DM when compared to controls as 84 μmol/L (p<0.0001). The TOS levels were increased in diabetes but not significantly. The mean TOS value was 10.4 μmol H\(_2\)O\(_2\) equivalent/l in control subjects in comparison to 11.9 in diabetics (p<0.086). Furthermore table 2 shows, significant correlation between HbA1c and AOPP when HbA1c level > 7.5%. (r=0.59p<0.001y =74.71+9.26x).

**Discussion**
The results of our study have shown that there is a significant increase in mean HbA1c and AOPP levels in diabetes in comparison to controls. Furthermore, significant positive correlation
between HbA1c and AOPP has also found in our study when HbA1c level >7.5%. The TOS levels were increased in diabetes but not significantly. The findings in our study keep in tract with the observations found by Ptwowar A(13) who described that AOPP formation is induced by intensified glyco-oxidation process, oxidant antioxidant imbalance and coexisting inflammation. Similar findings were also observed by Pan et al(2010)(27) and Fathy et al (2009)(28). Gil del valy(29) and Fathy et al(28), found correlations between AOPP and HbA1c. Furthermore Fathy et al(28) found correlations of AOPP with microvascular complications of T2DM. Kostolonska J(6) observed the significant correlations between HbA1c and AOPP in DM 1patients of poor glycemic controls. Kalousova also suggested that the increase of AOPP was more pronounced in T2DM than in T1DM. (P<0.001 in T2DM vs healthy controls, p<0.05 in T1DM vs healthy controls) and AOPP is a better parameter than AGE. In this context, we found significant correlations between HbA1c and AOPP at our region.(when HbA1c>7.5%) Previous studies also observed that AOPP is a good marker for progression of diabetic Nephropathy(6). Sharada HM(30) observed that AOPP increased progressively and significantly with the growth of albuminuria(p<0.01). A significant positive correlation was also found by him between plasma level of AOPP and both of (S) creatinine and Albumin creatinine ratio (ACR).

Zi Ziang Ng et al(14) found higher AOPP levels in diabetic retinopathy patients. From our review we can assume that at our region progression to diabetic nephropathy as well as retinopathy may appear earlier if HbA1c >7.5%.

In our study TOS level was not increased significantly in diabetes. Recently, numerous stable end products of oxidative stress have been identified and these include the AOPP(14). From our result we can assume that AOPP is a better marker than TOS in diabetes. AOPPs challenge also impaired insulin signalling. Oxidative stress has been proposed already as a causative factor of T2DM and responsible for its complications(3,4). Those observations may contribute the correlation of HbA1c >7.5% and AOPP. Accumulation of AOPP can cause further deterioration of hyperglycemia.

Estimation of AOPP as well as HbA1c may be useful to predict the risk of development of diabetic complications(6). AOPP can be a useful marker to estimate the degree of protein damage in diabetic patients(32).

Furthermore AOPP is not age and disease duration dependent unlike HbA1c(30). Some have hypothesised that there was a greater frequency and magnitude of glycaemic excursions in the conventionally treated patients compared with patients in the intensive treatment group, and that this increased glycaemic variability generated more ROS, leading to vascular damage(4).

Based on the results of our study and considering all the above facts we can consider AOPP estimation is very much beneficial in T2DM particularly when HbA1c is >7.5% at our region for prediction of diabetic complications. AOPP have their own particular biological proprieties, similar to those of AGEs, and also bind to the same receptor, i.e. RAGE(12). There is substantial evidence to support that the binding of AGE to its receptor (RAGE) is involved in the development of microvascular complications(33). According to Kalousova et al, AOPP shares common biological effect exerted by AGE, including interaction with RAGE which ultimately leads to neo-vascularisation that could result in DR(5). Zi Ziang ng, stated that, the soluble RAGE (sRAGE), a RAGE isoform lacking the transmembrane domain, is a recently discovered naturally occurring inhibitor of AGE-RAGE mediated pathological effects. According to him, the increased plasma sRAGE level in diabetic non retinopathy(DNR) and DR patients could be viewed as a protective reaction to counterbalance, at least partly, the elevated plasma pentosidine(AGE) level.
AOPP accumulation may be a therapeutic target at least for the prevention and delaying the progression of renal complications in diabetics\(^{12}\). Furthermore, medications targeted to increase the sRAGE level in plasma can also prevent the binding the AOPP with RAGE, to reduce the microvascular complications of T2DM.\(^{14}\)

**Conclusion**

Oxidative stress has been implicated in the progression of long-term diabetes complications, including microvascular and macrovascular dysfunction.

We have measured AOPP and TOS in type 2 diabetes and also tried to assess the correlation between AOPP and TOS with \(\text{HbA}_{1c}\). We observed that AOPP has increased significantly in type 2 diabetes but increase of TOS was not significant. Increase in AOPP also positively correlated with \(\text{HbA}_{1c}\) when \(\text{HbA}_{1c}\) is above 7.5% in our study. AOPP accumulation can further deteriorate hyperglycemia. So we recommend to measure the AOPP particularly if \(\text{HbA}_{1c}\) is >7.5%.

In view of the socio-economical status of our country, measurement of AOPP is beneficial as it is simple and not too costly and can be used as a therapeutic target of the physicians to challenge the chronic complications of diabetes.

**Acknowledgements**

The authors acknowledge Professor Mohan Mondal (Professor and HOD of Biochemistry, NRS Medical College) for his assistance and guidance in this research.

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**Table 1 : HbA\(_{1c}\), AOPP and TOS levels in control and type 2 diabetes patients**

<table>
<thead>
<tr>
<th>Study Population</th>
<th>Statistics</th>
<th>(\text{HbA}_{1c})%</th>
<th>(\text{AOPP}(\mu\text{mol/l}))</th>
<th>(\text{TOS(}\mu\text{mol H}_2\text{O}_2\text{ equiv/l}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Control)</td>
<td>Mean</td>
<td>5.45</td>
<td>84.23</td>
<td>10.44</td>
</tr>
<tr>
<td>(n = 28)</td>
<td>SD</td>
<td>0.2755</td>
<td>12.39</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>SEM</td>
<td>0.052</td>
<td>2.3415</td>
<td>0.3232</td>
</tr>
<tr>
<td>Group II (T2DM)</td>
<td>Mean</td>
<td>8.296</td>
<td>139.6</td>
<td>11.94</td>
</tr>
<tr>
<td>(n = 50)</td>
<td>SD</td>
<td>1.3019</td>
<td>28.85</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>SEM</td>
<td>0.18</td>
<td>4.08</td>
<td>0.321</td>
</tr>
<tr>
<td>p-value</td>
<td>Significance</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.086</td>
</tr>
</tbody>
</table>
Table 2: Correlation between HbA\textsubscript{1c} and AOPP levels in type 2 diabetes

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n=28)</th>
<th>Sub-Group-A(n=26)</th>
<th>Sub-Group-B(n=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>coefficient (r):</td>
<td>0.3469</td>
<td>0.49984</td>
<td>0.5976</td>
</tr>
<tr>
<td>HbA\textsubscript{1c} &amp; AOPP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-value</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.086</td>
</tr>
</tbody>
</table>

Fig. 1: Correlation between HbA\textsubscript{1c} & AOPP in Sub-group B(HbA\textsubscript{1c} > 7.5%) Type 2 Diabetes Mellitus patients
Reference


Introduction
Malaria along with six other diseases viz. diarrhea, pneumonia, tuberculosis, measles, hepatitis B and HIV/AIDS account for 85% of Global infectious disease burden1. Thirty-six percent of global population i.e. 2020 million in 107 countries and territories situated in the tropical and subtropical regions are at the risk of malaria. Of the 2.5 million reported cases of malaria in the South East Asia, India alone contributes about 70% of the total cases1. Plasmodium falciparum is the most deadly among the five Plasmodium species that cause human malaria and it is the world’s second biggest killer after tuberculosis. It is estimated that 3,000 children under the age of five years fall victim to malaria each day. Around 40% of the world population are at risk2. Severe malaria kills over a million people every year. The annual death toll can be as high as one in a 100 children under the age of five3,4.

Plasmodium falciparum infection is frequently complicated with multi-organ failure along with or without metabolic dearrangement5. Cerebral malaria, un-arousable coma, not attributable to any other cause, is a specific type of severe malaria4 that even with correct treatment can have a morality rate approaching 20%6.

A HOSPITAL BASED RETROSPECTIVE CLINICAL EVALUATION OF COMBINED ANTI-MALARIAL THERAPY ON THE OUTCOME OF PLASMODIUM FALCIPARUM MALARIA CASES IN YOUNG HOSPITALIZED TRIBAL CHILDREN OF TRIPURA: A THREE YEARS STUDY

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Abstract
This study is aimed at the comparative evaluation of drug in practice in terms of therapeutic use of antimalarial drugs against plasmodium falciparum (p.f.) positive malaria cases and to compare the efficacy of artesunate and quinine in the treatment of p.f. positive malaria in hospitalized children. All the p.f. positive malaria cases who were admitted from May 2008 to Oct 2011 were included in this study. The prescribing pattern of anti-malarial in these cases and its clinical outcome like mortality and morbidity patterns includes the fever remission time and total hospital stay was assessed from the case sheets. Out of the total 264 clinically suspected malaria cases, 121(46%) cases were p.f. positive and among them 31(25.6%) cases received quinine based combination therapy and rest 90(74.4%) received artesunate based combination therapy. Among the 121 p.f. positive cases 69(57%) were admitted with pronounced symptoms of malaria (severe malaria). Severe anaemia and cerebral malaria were the two commonest presentation of severe falciparum malaria. There was no statistically significant difference of clinical outcome between the both clinical groups in the form of mortality, time for remission of fever (fever clearance time) or total hospital stay. Randomized clinical trial with a large sample size is necessary to compare the efficacy of both the quinine based and the artesunate based combination therapy for plasmodium falciparum Malaria.
Over the years quinine has been the drug of choice for treating severe malaria in children, but despite its high efficacy the case fatality rate for severe falciparum malaria in children can be as high as 40%. Additionally, in recent years there have been concerns that the efficacy of quinine is declining in some parts of South East Asia and quinine resistance cases has been documented in Africa. This led to the launch of a series of trials to find newer effective drugs that are suitable alternatives to quinine in terms of efficacy(7).

In anti-malarial combination therapy, the combination is often more effective and delay the emergence of resistance. The each component of the combination therapy must independently be sufficiently efficacious in treating malaria(8).

Currently two classes of drugs are available for the parenteral treatment of severe malaria: the cinchona alkaloids (quinine and quinidine) and the artemisinin derivatives (artesunate, artemether and artemotil)(8).

The clinical resistance to quinine therapy has been noticed sporadically in Southeast Asia and Western Oceania. Therefore, since the last two decades this drug has been used in combination with tetracycline or doxycycline to enhance its effectiveness. In India resistance has emerged against quinine in northeastern states of India and Kolar district in Karnataka(9).

Intravenous artesunate is the drug of choice for adults with severe falciparum malaria, particularly if acquired in Asia. But literature, on earlier studies did not identify sufficient data to make firm conclusions about the treatment of children or the effectiveness of intramuscular artesunate administration. There is an urgent need to compare the effects of artesunate with quinine in children with severe falciparum malaria(10).

In India, malaria is reported from many states, but several deaths, amounting to epidemic proportion, reported every year from different parts of north-east India. Chloroquine-resistant malaria and decreased sensitivity to other anti-malarial drugs has been documented(11).

The North eastern States of India are highly endemic area for malaria in terms incidence and transmission, and the control of P. falciparum malaria poses a great challenge to the society hampering manpower loss which is reflected by lower scale of development of this part of the country in comparison to the rest of the country(12).

The state of Tripura in the North Eastern Region of India is surrounded by Bangladesh except in the north. The areas adjacent to the Indo-Bangladesh border are at risk of malaria outbreak due to inadequate health infrastructure and lack of vector control operations(13).

To the best of our knowledge, there is no published data reported from Tripura about the comparison of artesunate and quinine in the treatment of severe falciparum malaria in children. So there is need to compare the efficacy of artesunate with quinine in children of Tripura especially tribal children from rural area suffering from severe falciparum malaria so that a cheap drug like quinine is not abandoned completely.

Agartala Govt. Medical College and GBP Hospital is the only tertiary health care centre in Govt. sector with paediatric intensive care unit, where a good number of severe malaria patients are referred from various parts of the state and treated every year. We carried out this retrospective study from the hospital record and this may be a useful guide to design the randomized control trial (RCT) in future to compare the efficacy of Quinine with Artesunate based combination therapy in children especially in this part of the country.

Materials and Methods
It was about three and half years retrospective study and all the tribal children suffering from p.f. Malaria and admitted in paediatric ward from May 2008 to Oct 2011 were included for this study. Outcome of two treatment modalities, one
group which received quinine with clindamycin (when age is less than 8 yrs) or with tetracycline (when age is ≥8 yrs) was compared with the other group which received artesunate with clindamycin (when age is less than 8 yrs) or with tetracycline (when age is ≥8 yrs). The current practice of treating the young children suffering from severe malaria and its clinical outcome was included in this study and assessed from the case sheets which were collected from the Medical Record section of the Agartala Govt. Medical College and GBP Hospital. The cases were enrolled in this study either as severe or as non severe was as per the entry in the bed head ticket diagnosed by the clinicians. Data collected and recorded on a pre-tested format that contained information on the demography of the patients, type of anti-malarial drugs used, mortality, fever clearance time (fever recovery time), total hospital stay, adverse effects, neurological sequel and any adjuvant therapy administered. Complete free from fever were considered as recovery.

The collected data were analyzed for statistical significance to compare the clinical outcome of p.f. malaria cases that were either treated by intravenous quinine or by intravenous artesunate based combination therapy (ACT).

The data were expressed as mean±standard error of mean (SEM) and in percentage of outcome between the groups. Statistical comparison between the different treatment groups were done using either student’s t test or chi-square test whichever applicable and P <0.05 was considered significant.

Results and observations
Out of the total 264 clinically suspected malaria cases, 121(46%) cases were p.f. positive and were included for this study. Out of the total 121 cases 31(25.6%) cases received quinine based combination therapy and rest 90(74.4%) received artesunate based combination therapy. Among the 121 p.f. positive cases, 69(57%) cases were suffering from severe malaria and rest 52(43%) were non severe p. f. positive cases. Out of total 69 severe malaria cases, 16(23.2%) were treated with intravenous quinine based combination therapy and rest 53(76.8%) were treated with artesunate based combination therapy. The demographical distributions of severe falciparum malaria cases were shown in Table1. There was no significant demographical variation between the two groups. Maximum of the p. f. positive patients (99%) were from rural area.

Table 2 shows the age, district wise and different native ethnic group distribution of the cases received two modalities of treatment. There was no significant difference between the two treatment groups in different age group, in different native ethnic group and in patients from different districts.

Different diagnostic methods used for diagnosis of 121 p.f. malaria under this study population were peripheral smear slide method (60 cases 49.6%), QBC (Fluorescence microscopy) (10 cases 8.2%) and Rapid diagnostic tests (51 cases 42.1%). There was no significant difference in the choice of methods, for malaria diagnosis.

In table 3 the different clinical presentations of p.f. positive severe malaria cases were shown. Out of the total 69 severe malaria cases, 24 (34.8%) and 42 (60.9%) presented with cerebral malaria and severe anaemia respectively. 16 (23.2%) of total severe malaria cases received quinine based combination therapy and 53 (76.8%) of total severe malaria cases received artesunate based combination therapy.

Table 4 shows the clinical outcome profile in patients treated with two modalities of treatment. There were no significant differences in fever recovery time and total duration of hospital stay between the two treated groups. Complete free from fever were considered as recovery. 41.3% and 17.4% of the total p.f. positive cases received blood transfusion and anticonvulsant therapy respectively. There was no significant variation regarding receiving blood transfusion and anticonvulsants between the two treatment groups.
Discussion
Malaria is one of the commonest potentially fatal infections in the world with high incidence in South East Asia region\textsuperscript{(14)}. In India malaria is reported from many states, but deaths are mostly reported every year from different parts of northeast India\textsuperscript{(11)}. In recent years there have been serious concerns over the declining efficacy of quinine in some parts of South East Asia and quinine resistance also has been documented in Africa. This led to the launch of a series of trials to find out the drugs that are suitable alternatives to quinine\textsuperscript{(7)}. There is an urgent need to compare the efficacy of artesunate with quinine in children with severe falciparum malaria\textsuperscript{(10)}. Since till date there is no published data / records from Tripura about the comparative efficacy of artesunate with quinine for the treatment of severe falciparum malaria in children are available, so need of such study was a demanding one. Randomized Control Trials (RCT) can be designed if any clue can be identified from such type of retrospective study.

The malaria cases are detected throughout the year in the state but higher incidence is reported during May to October\textsuperscript{(11)}. Keeping conformity with higher incidence of malaria cases during this period, for this retrospective study period was selected from May, 2008 to Oct, 2011.

The baseline characteristics of the two drug groups were comparable. The mean age of children suffering from malaria in two treatment group was around 5.2 years to 5.6 years and is comparable with the observations of Huda SN et al 2003 14.

Malaria was diagnosed by standard methods of diagnosis like peripheral smear examination both thick and thin, QBC (Fluorescence microscopy) and Rapid diagnostic test. There was no significant difference regarding the choice of diagnostic methods.

Malaria cases were found in all ethnic group of native community of Tripura and there was no significant difference in the incidence of malaria cases. Though it was observed that the number of malaria cases from North Tripura and Dhalai district, treated in AGMC were less but it may not reflect the true incidence as both the districts are far away from AGMC and might been treated in some other well equipped institutions.

In the present study it was observed that intravenous artesunate based combination therapy was preferred (74.4% ) over quinine based combination therapy (23.6%) for treatment of p.f. positive cases by the clinicians and this may probably be due to some reported concern on the declining efficacy of quinine, both in our country and the South East Asian and African countries\textsuperscript{7}. But there was no significant difference in recovery time (fever remission time) and total hospital stay in both the treatment group and is comparable to the findings of other studies\textsuperscript{(7,10,11)}.

Mortality rate in the patients receiving artesunate based combination therapy was marginally less (13.2% vs 18.8%) than the patients received quinine based combination therapy but this difference is statistically insignificant. This observation is in consistent with the earlier studies from other part of the world\textsuperscript{(7,10,11)}.

This study revealed that the commonest presentation of severe falciparum malaria was with severe anaemia followed by impaired consciousness/coma then by repeated convulsion. This is in contrary to the observations of other studies where cerebral malaria is the commonest presentation\textsuperscript{(7,11)} of severe falciparum malaria and this probably may be due to the fact that the 99% of this study population are of rural native origin who are mostly from lower socio economic family and suffering from nutritional anaemia and developed pronounced complication with malaria. There was no report of adverse drug reactions among the both treated group patients. Due to insufficient case record data, parasite clearance time and coma recovery time and severe falciparum malaria presented with hypoglycaemia, hyperpyrexia, metabolic
acidosis could not be assessed. Though the clinicians of this institution preferred artesunate based combination therapy over quinine based combination therapy for treatment of p.f. positive malaria, but this retrospective study does not indicate any significant advantage of artesunate based combination therapy over quinine based combination therapy. Hence a larger randomized control trial and firm clinical evidence is necessary to make a firm conclusion about the comparative efficacy of artesunate based combination therapy with quinine based combination therapy among the children of this malaria endemic zone of the country so that a cheap and useful drug like Quinine is not rejected by the clinicians.

Acknowledgement
Authors are grateful to the Principal and Medical Record section of Agartala Govt. Medical College, Agartala, Tripura India, for providing necessary facility for this retrospective study.

Reference
### Table 1: Comparative demographic profile of the p.f. positive malaria cases that were treated either with Quinine or ACT in AGMC from May 2008 to Oct 2011

<table>
<thead>
<tr>
<th>Variables</th>
<th>Quinine based combination therapy group</th>
<th>Artisunate based combination therapy group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total p.f. positive Cases (N = 121)</td>
<td>31 (25.6%)</td>
<td>90 (74.4%)</td>
</tr>
<tr>
<td>Severe malaria out of p.f. Positive Cases (N = 69)</td>
<td>16 (23.2% total severe cases)</td>
<td>53 (76.8% total severe cases)</td>
</tr>
<tr>
<td>Non-Severe p.f. Positive Cases (N = 52)</td>
<td>15 (28.8%)</td>
<td>37 (71.2%)</td>
</tr>
<tr>
<td>Age in yrs. (Mean ± SEM)</td>
<td>5.2 ± 0.7</td>
<td>5.6 ± 0.3</td>
</tr>
<tr>
<td>Weight in kg (Mean ± SEM)</td>
<td>14.4 ± 1.3</td>
<td>15.8 ± 0.63</td>
</tr>
<tr>
<td>Sex (M:F)</td>
<td>15 : 16</td>
<td>46 : 44</td>
</tr>
<tr>
<td>Address (Urban : Rural)</td>
<td>0 : 32</td>
<td>1 : 88</td>
</tr>
</tbody>
</table>

### Table 2: Age, district wise and ethnic distribution of the p.f. positive malaria cases that were treated either with Quinine or ACT in AGMC from May 2008 to Oct 2011

<table>
<thead>
<tr>
<th>Variables</th>
<th>Quinine based combination therapy group</th>
<th>Artisunate based combination therapy group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age 0 to &lt;1 yrs</td>
<td>5 (4.1%)</td>
<td>3 (2.5%)</td>
</tr>
<tr>
<td>1-3 yrs</td>
<td>7 (5.8%)</td>
<td>24 (19.8%)</td>
</tr>
<tr>
<td>&gt;3 to 6 yrs</td>
<td>9 (7.4%)</td>
<td>26 (21.5%)</td>
</tr>
<tr>
<td>More than 6 yrs</td>
<td>10 (8.3%)</td>
<td>37 (30.6%)</td>
</tr>
<tr>
<td>District Wise Distribution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dhalai</td>
<td>3 (2.5%)</td>
<td>8 (6.6%)</td>
</tr>
<tr>
<td>North Tripura</td>
<td>0</td>
<td>1 (0.8%)</td>
</tr>
<tr>
<td>South Tripura</td>
<td>5 (4%)</td>
<td>22 (18.2%)</td>
</tr>
<tr>
<td>West Tripura</td>
<td>23 (19%)</td>
<td>59 (48.8%)</td>
</tr>
<tr>
<td>Different ethnic Tribal groups</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tripuri</td>
<td>26 (21.5%)</td>
<td>63 (52.1%)</td>
</tr>
<tr>
<td>Halam</td>
<td>2 (1.7%)</td>
<td>9 (7.4%)</td>
</tr>
<tr>
<td>Reang</td>
<td>2 (1.7%)</td>
<td>13 (10.7%)</td>
</tr>
<tr>
<td>Chakma</td>
<td>1 (0.8%)</td>
<td>4 (3.3%)</td>
</tr>
<tr>
<td>Mog</td>
<td>0</td>
<td>1 (0.8%)</td>
</tr>
</tbody>
</table>
Table 3: Different presentations of the severe p. f. malaria cases that were treated either with Quinine based combination or Atisunate based combination therapy in AGMC from May 2008 to Oct 2011

<table>
<thead>
<tr>
<th>Types</th>
<th>Quinine based combination therapy</th>
<th>Artisunate based combination therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>p.f. positive severe malaria cases (N = 69)</td>
<td>16 (23.2%)</td>
<td>53 (76.8%)</td>
</tr>
<tr>
<td>Impaired consciousness/coma N = 24 (34.8%)</td>
<td>7 (10.2%)</td>
<td>17 (24.6%)</td>
</tr>
<tr>
<td>Repeated generalized convulsions N = 21(30.4%)</td>
<td>4 (5.8%)</td>
<td>17 (24.6%)</td>
</tr>
<tr>
<td>Severe anaemia (Hb &lt;5mg/dl) N = 50 (41.3%)</td>
<td>9 (13%)</td>
<td>41 (59.4%)</td>
</tr>
<tr>
<td>Algid Malaria = 1 (1.4%)</td>
<td>0</td>
<td>1 (1.4%)</td>
</tr>
<tr>
<td>Anaemia with Renal Failure N= 2 (2.9%)</td>
<td>1(1.4%)</td>
<td>1(1.4%)</td>
</tr>
<tr>
<td>Jaundice (Serum Bilirubin &gt;3 mg/dl) N=1 (1.4%)</td>
<td>0</td>
<td>1(1.4%)</td>
</tr>
<tr>
<td>Abnormal bleeding and Disseminated intravascular coagulation</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pulmonary oedema / acute respiratory distress syndrome N=1 (1.4%)</td>
<td>0</td>
<td>1(1.4%)</td>
</tr>
</tbody>
</table>
Table 4: Comparative clinical outcome profile of the p.f. malaria cases that were treated either with Quinine or ACT in AGMC from May 2008 to Oct 2011

<table>
<thead>
<tr>
<th>Types</th>
<th>Quinine based combination therapy (No of cases)</th>
<th>Artisunate based combination therapy (No of cases)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total p.f. positive cases (N = 121)</td>
<td>31 (25.6%)</td>
<td>90 (74.4%)</td>
</tr>
<tr>
<td>p.f. positive severe malaria cases N = 69 (57% of total p.f. positive cases)</td>
<td>16 (23.2% total severe cases)</td>
<td>53 (76.8% total severe cases)</td>
</tr>
<tr>
<td>Mortality N= 10 (8.3%)</td>
<td>3 (9.7% within the group)</td>
<td>7 (7.8% within the group)</td>
</tr>
<tr>
<td>Mean time of death since admission (hrs) (Mean ± SEM)</td>
<td>9.2 ± 7.5</td>
<td>23.6 ± 13.7</td>
</tr>
<tr>
<td>Recovery time (Fever clearance Time) in days (Mean ± SEM)</td>
<td>2.5 ± 0.3</td>
<td>3.2 ± 0.2</td>
</tr>
<tr>
<td>Total duration of hospital Stay in days (Mean ± SEM)</td>
<td>6 ± 0.7</td>
<td>6.5 ± 0.3</td>
</tr>
<tr>
<td>Blood transfusion Received N = 50 (41.3% of total pf positive cases)</td>
<td>9 (29% within the group)</td>
<td>41 (45.5% within the group)</td>
</tr>
<tr>
<td>Any anticonvulsant therapy N=21 (17.4% of total pf positive cases)</td>
<td>4 (12.9% within the group)</td>
<td>17 (18.9% within the group)</td>
</tr>
<tr>
<td>Any bleeding episodes</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Any suspected adverse drug reactions</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Corrigendum

The name of authors & affiliation for the paper titled with **SERUM URIC ACID LEVEL AS A PREDICTOR OF FETAL OUTCOME IN PREGNANCY INDUCED HYPERTENSION**, published in IJMB, Volume 16, No. 2 : 2012 is as follows:

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²Department of Biochemistry, Sir T General Hospital, Bhavnagar, Gujarat, India
³Department of Obs. & Gynecology, M P Shah Medical College, Jamnagar, Gujarat, India
⁴Department of Biochemistry, GMERS Medical College, Valsad, Gujarat, India

But it should be

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We are sorry for the mistake.

Editorial Board
SELENIUM AND VITAMIN E STATUS IN PATIENTS WITH ALCOHOLIC CIRRHOSIS

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Abstract

The purpose of the study is to show that oxidative stress and lipid peroxidation plays a significant role in the pathogenesis of ethanol-induced liver injury. Heavy drinkers are deficient in Selenium, which is required for the activity of Glutathione Peroxidase and antioxidant vitamins A, C and E. This study aims at assessing the antioxidant status of the patients with alcoholic cirrhosis and its correlation with severity of liver damage. The study population includes 50 healthy volunteers who served as control and 50 cases of alcoholic cirrhosis. The blood samples were analysed for selenium, vitamin E and Liver Function Tests viz. bilirubin, aspartate transaminase, alanine transaminase and albumin. The mean and standard deviation estimated for cases vs controls were Selenium (0.68 ± 0.05 vs 1.03 ± 0.04) and vitamin E (19 ± 0.7 vs 29.7 ± 1.2). Selenium and Vitamin E were significantly lower in cases when compared to controls.

Introduction

Alcohol is consumed by a large percentage of world’s population and a moderate proportion of them develop clinically significant liver disease. Alcohol is the common cause of liver damage worldwide. There is a significant role of oxidative stress and lipid peroxidation in the pathogenesis of ethanol-induced liver injury. Ethanol consumption results in depletion of endogenous antioxidant capabilities. Depletion of mitochondrial GSH (Reduced Glutathione) precedes and promotes the progression of alcoholic liver injury. Heavy drinkers are deficient in Selenium which is required for the activity of Glutathione Peroxidase and antioxidant vitamins A, C and E(1). This study aims at assessing the antioxidant status of the patients with alcoholic cirrhosis by analyzing selenium and vitamin E levels and to assess its correlation with severity of liver damage.

Materials and Methods

This is an age and sex matched case-control study conducted during the period of March 2013 to September 2013. Prior approval was obtained from the institutional ethical committee. The study population includes two groups; 50 healthy volunteers who served as controls and 50 cases of alcoholic cirrhosis. The cases were recruited from Medical Gastroenterology department, Stanley Medical College and Hospital.
Inclusion criteria
Patients with ethanol related decompensated liver disease with portal hypertension were included in the study. All patients had varying grades of oesophageal varices as identified by GI endoscopy.

Exclusion criteria
1. Non alcoholic liver disease
2. Patients on vitamins and minerals supplementation during the past one year.
3. Patients with positive serology for Hepatitis B and C.
4. Patients with other coexisting medical or surgical illness like CAD, stroke, diabetes etc.

Sample collection
Random venous blood sample of 5 ml was collected. The serum was separated and analysed for Selenium, Vitamin E, Bilirubin, Aspartate transaminase, Alanine transaminase and Albumin.

Determination of Selenium
The sample was acid digested before analysis. The sample of 500μl was taken in a porcelain dish and 1.5 ml of nitric acid : perchloric acid mixture (ratio 5:1) was added and heated on a sand bath until complete ashing. The ashed sample was reconstituted with 2 ml of 0.2% nitric acid. The reconstituted sample was centrifuged for 10 minutes at 2500 rpm and the clear supernatant was transferred into another glass tube. The digested sample was submitted for analysis.

Quantitative estimation of selenium was done by Graphite Furnace- Atomic Absorption Spectrophotometer with Zeeman background correction (Perkin Elmer Analyst 700) in the Department of Biochemistry, Shankara Nethralaya.
Selenium in vapourized sample absorbs energy at wavelength of 196.3 nm. Absorbance at this wavelength is specific for selenium and is proportional to its concentration$^{[2]}$.

Reference interval: Adult - 0.8 – 2 μmol/l

Estimation of Vitamin E
Serum vitamin E was estimated by spectrophotometric method using bathophenanthroline and is based on the reducing property of vitamin E$^{[3]}$. Alpha Tocopherol being a reducing agent can convert ferric ion into ferrous ion which can then combine with the chelating agent, Bathophenanthroline (BA) to produce pink coloured Ferrous- BA complex. The absorbance of this complex is measured at 536 nm which is proportional to the concentration of α Tocopherol in the reaction. Orthophosphoric acid is used to inhibit the interference caused by other reducing agents such as β carotene and glutathione and hence increases the specificity of the assay.
Reference range : Adults : 12 - 42 μmol/L

Estimation of Total Bilirubin was done by Diazo Method of Pearlman & Lee. Aspartate transaminase (AST) by IFCC Method$^{[4]}$, Alanine transaminase by modified IFCC method and Albumin by Bromocresol green dye binding method.

Result and Statistical Analysis
The statistical analysis was done using Graph Pad Prism 6 statistical software. The distribution of age among the control group and cases were as shown in table no.1. Mean and standard deviation were estimated for each group (cases and controls). Mean values were compared using student independent ‘t’ test (Table no.2). Pearson’s correlation analysis was done to find out the relationship between Selenium and Vitamin E (Table no.3). The relationship is graphically represented by scatter diagram. Correlation analysis was also done for serum Bilirubin with selenium and vitamin E (Table no. 4)

Discussion
The present study demonstrates the defects in antioxidant status of patients with alcoholic
Selenium and Vitamin E Status in Patients With Alcoholic Cirrhosis

A decrease in plasma Selenium has been found previously by Johansson et al\(^5\). It may be due to low intake, absorption or metabolism in alcoholics. Plasma selenium was found to be decreased in alcoholic cirrhosis patients but not among alcoholics in abstinence. This shows that selenium deficient state in chronic alcoholics promotes the progression of liver cirrhosis. Sakena H Rashed et al has found the Vitamin E levels to be significantly reduced in patients with cirrhotic liver\(^6\). In the present study serum Selenium and Vitamin E levels were found to be significantly lower in alcoholic cirrhosis patients compared to the control group (Table no 2). The Karl Pearson’s correlation coefficient between serum selenium and vitamin E showed a significant and positive correlation (Table no 3). Serum Bilirubin shows a significant and negative correlation with selenium and vitamin E levels in cases. P Clot et al has found the markers of oxidative damage such as plasma lipid hydroperoxide and erythrocyte malondialdehyde to be 4-5 fold higher in alcohol intake of more than 100g/day\(^7\). This shows an altered redox status in these individuals with several derangements in the natural antioxidant defence mechanisms.

Further validation studies are needed to evaluate the effects of selenium and vitamin E supplementation in patients with alcoholic liver disease in attenuating the progression of hepatitis to cirrhosis. The huge disease burden necessitates the urgent need for initiation of nutritional intervention trials in alcoholic hepatitis patients to find out the benefits in terms of reduction of morbidity and mortality. Although cornerstone of therapy in alcoholic hepatitis is abstinence, there is increased risk of recidivism in patients who attempt to cut back but not stop drinking altogether\(^8\). And even if the patient becomes abstinent, the risk of developing cirrhosis is very high\(^9\). So nutritional intervention if proven beneficial, it may have a significant role in treatment of alcoholic hepatitis in future.

**Conclusion**

The present study shows decreased levels of antioxidant nutrients in patients with alcoholic cirrhosis. Selenium and vitamin E supplementation at an early stage of alcoholic liver disease may improve the outcome and long term prognosis in these patients.

**Table 1: Age distribution among the cases and controls**

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean age (yrs)</th>
<th>SD</th>
<th>Student ‘t’ test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case</td>
<td>50</td>
<td>43.14</td>
<td>1.4</td>
<td>P=0.653</td>
</tr>
<tr>
<td>Control</td>
<td>50</td>
<td>42.18</td>
<td>1.6</td>
<td>Not significant</td>
</tr>
</tbody>
</table>

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\(^5\) Johansson et al, 2005
\(^6\) Sakena H Rashed et al, 2010
\(^7\) P Clot et al, 2011
\(^8\) Cornerstone of therapy in alcoholic hepatitis is abstinence
\(^9\) Risk of developing cirrhosis is very high
Table 2: Mean, Standard deviation and Test of significance of mean values between cases and controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± Standard deviation</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>Controls</td>
</tr>
<tr>
<td>Bilirubin (µmol/L)</td>
<td>128.2±22</td>
<td>10.4±0.51</td>
</tr>
<tr>
<td>AST (µkat/L)</td>
<td>1.6±0.25</td>
<td>0.37±0.01</td>
</tr>
<tr>
<td>ALT (µkat/L)</td>
<td>0.4±0.04</td>
<td>0.2±0.01</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>28±3</td>
<td>52±0.6</td>
</tr>
<tr>
<td>Selenium (µmol/L)</td>
<td>0.68±0.05</td>
<td>1.03±0.04</td>
</tr>
<tr>
<td>Vitamin E (µmol/L)</td>
<td>19±0.7</td>
<td>29.7±1.2</td>
</tr>
</tbody>
</table>

Table 3: Correlation of serum Selenium and Vitamin E in Alcoholic Cirrhosis patients

<table>
<thead>
<tr>
<th>Variables</th>
<th>Pearson's correlation coefficient (r)</th>
<th>Significance (p)</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selenium vs Vitamin E</td>
<td>0.785</td>
<td>&lt;0.0001</td>
<td>Significant and positive correlation</td>
</tr>
</tbody>
</table>

Table 4: Correlation of Bilirubin with Selenium and Vitamin E in alcoholic cirrhosis patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pearson's correlation coefficient (r)</th>
<th>Significance (p)</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin E vs Bilirubin</td>
<td>-0.529</td>
<td>&lt;0.0001</td>
<td>Significant and Negative correlation</td>
</tr>
<tr>
<td>Selenium vs Bilirubin</td>
<td>-0.425</td>
<td>0.002</td>
<td>Significant and Negative correlation</td>
</tr>
</tbody>
</table>
Reference


Test Aims to Identify Heart Attacks Before They Occur

A point-of-care assay to detect a heart attack about to happen, which would give clinicians time to intervene before myocardial damage occurs, is being developed by genomics researchers. They found 11 genes that were either significantly up regulated or significantly down regulated in patients with AMI, compared with control subjects. Their panel had a perfect discriminative ability, as indicated by the 1.0 area under the curve (AUC) of receiver operating characteristics (ROC).

"We thought that was pretty awesome, but seemingly unbelievable, so we went back and recruited another 25 healthy volunteers, in addition to the 23 crashing patients with acute myocardial infarction. Using this 11-gene model, we were able to see an AUC of ROC analysis of 0.99, which we felt was quite fantastic," The researchers reported.

Many of the genes included in the panel have already been recognized as being involved in inflammatory signaling and vesicular trafficking, such as endothelin-1, an endothelium-specific protein implicated in the risk for cardiac disease.

The team also tested the concept on whole blood samples from AMI patients and healthy control subjects, using quantitative real-time polymerase chain reaction (PCR) on a test panel of seven of the genes, and found an AUC of 0.998.

The seven-gene test was a little less robust when they tested it on a third cohort of patients who had stable coronary disease. Nearly two-thirds of that control set had previously undergone coronary angioplasty with stenting or coronary artery bypass graft, and one-quarter had atrial fibrillation. For this test run, the AUC was 0.85 — not perfect, but still quite impressive.

"We moved from having to use immunomagnetic separation techniques and microarrays of thousands of genes to a real-time PCR of just seven genes from whole blood, something that could potentially be moved into a real clinical setting for relevance," the researchers explained.

Obesity gastric bands may cause more complications than weight loss

Almost half of patients undergoing gastric banding for obesity need to have the device removed, according to a study published in the Archives of Surgery.

About 60 percent of the 82 patients with the device, Allergan Inc.’s Lap–Band, followed over 12 years or more needed additional operations. The minimally invasive surgery led to weight loss in roughly 18 percent of the patients. About 40% of the 82 patients had major complications. Another 22 percent experienced relatively minor complications, and almost 50 percent had to have the bands entirely removed. In all but a few cases, inadequate weight loss or device breakdown was the reason for band removal. The mean reduction in body mass index was 7.8 points.