BIOCHEMICAL AND SEROLOGICAL PROFILING OF SANDHI SHOOLA (ARTHRALLGIA) PATIENTS OF AYURVEDA HOSPITAL

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ABSTRACT
Sandhi shoolla refers to the joints pain where there is pain with or without inflammation and can be caused by injury or a number of autoimmune disorders such as rheumatoid arthritis and gout. Ayurveda system of medicine believes this as Sandhi shoolla condition in which there is difficulty in walking and running and based on the physical examination, it is categorized into major types i.e. Aamvata (AV), Sandhivata (SV) and Vatarakta (VR). However, biochemical and serological investigations for Sandhi shoolla were not cited in ancient Ayurveda literature. A retrospective study in which biochemical and serological investigations such as uric acid (UA), Ca\(^{2+}\) and rheumatoid arthritis factor (RAF), C-reactive protein (CRP), antistreptolysin-O (ASO) were carried out respectively in 50 Sandhi shoolla patients of Ayurveda hospital in Kolkata. The individual information about clinical symptoms and diagnosis by the hospital professionals were recorded. UA and Ca\(^{2+}\) content in serum was determined by enzymatic and Cresolphthalin complexone kit methods respectively, however RAF, CRP and ASO were determined through latex agglutination method. Results showed that maximum number of patients (24) suffered from AV followed by SV (15) and VR (11). The range of the UA and Ca\(^{2+}\) were 2.6 – 18.0 mg/dl and 7.0 – 11.3 mg/dl respectively. The average UA content is maximum in case of VR, while no significant difference was found in Ca\(^{2+}\) content among the three Sandhi shoolla. Serological tests indicated maximum RAF positive samples in case of AV followed by SV patients, while CRP was positive in maximum patients of AV and SV. Results revealed a strong diagnostic measure for Sandhi shoolla and suggested that higher UA content and positive RAF should be a diagnostic measure in case of Vatarakta and Aamvata respectively. In conclusion, modern biochemical and serological investigations should be exercised in Ayurveda practice for diagnostic and validation with scientific justification strengthening the Ayurvedic principles.

Keywords: Arthralgia; rheumatoid arthritis factor; C-reactive protein, uric acid.

INTRODUCTION
Sandhi shoolla (Arthralgia) seems to be the most important disease encountered in Ayurveda hospital Out Patient Department (OPD). Sandhi shoolla refers to the pain with or without inflammation and can be caused by injury or a number of autoimmune disorders such as rheumatoid arthritis and gout. Ayurveda system of medicine believes that dosha involved in the arthralgia is Vata dosha, a condition in which there is difficulty in walking and running. Arthralgia targets the joints when they are in vitiated state and produces the disease called arthritis. Based on the symptoms and physical examination, it is categorized into major types i.e. Aamvata (AV), Sandhivata (SV) and Vatarakta (VR). When Vata dosha combines with the aama (indigestible material or bad toxins), it gets deposit in different joints of the entire body and produces a disease called as 'Aamvata'. It may be correlated as rheumatoid arthritis as per the modern medical science's classification and falls under autoimmune disease. Sandhivata is the type of Sandhi shoolla, which is generally, develops in elderly. This is characterized by chronic degeneration of the cartilages of the joints and thus labeled as degenerative joint disease. Ayurveda literature believes this to be Asthigata Vata or Sandhivata. Asthi means the bones and vata means the arthritis condition in which, Vata dosha is involved. When the Vata dosha gets disturbed and takes place deep into the bone, this situation arises. The vatarakta refers to the group of metabolic disorders in which sodium urate crystals get deposited in the tissues of the body and elevate the uric acid level in the blood. Ayurveda describes this situation as 'Vatarakta'. Vatarakta is the condition in which, the blood and Vata dosha both get vitiated and troubles an individual. The typical gouty condition catches the small joints of the body first. In Ayurveda understanding of the disease process has been given upper edge over the diagnosis of the diseases merely by its name. However, biochemical and serological investigations for Sandhi shoolla were not cited in ancient Ayurveda literature. Therefore development of workable diagnostic criteria seems to be important step in this regard. A specific criteria for the diagnosis of certain disease is also required for the epidemiological studies. Disease diagnosis in Ayurvedic hospital includes symptomatic multiple features in a cumulative fashion in description of a disease and remained a subjective phenomenon, which permits for a substantial flexibility in individual opinion regarding the disease and creating more confusion in disease understanding and treatment. There may be some symptoms which develop in course of time as the disease proceeds, while some symptoms may overlap with other and create confusion. The diagnostic confusion in Ayurveda further heightens by observing the fact of assembling the disease based upon the symptoms profiles and not upon the specific pathology. Many diverse disease are often grouped together just because of the common symptoms irrespective of the possible...
etiology and pathology. This kind of disease understanding is bound to make difficulty in disease management unless the biochemistry, pathologies and etiologies are more precisely understood. Therefore, the present study was conducted with the aim to establish a biochemical and serological profile of the Sandhi shoola (Arthralgia) patients.

**MATERIAL AND METHODS**

A diagnostic criteria was planned to be developed for Sandhi shoola patients in Department of Pathology and Biochemistry at National Research Institute for Ayurvedic Drug Development, Kolkata. A retrospective study in which biochemical and serological investigations such as uric acid (UA), Ca\(^{2+}\) and rheumatoid arthritis factor (RAF), C-reactive protein (CRP), antistreptolysin-O (ASO) were carried out respectively in 50 Sandhi shoola (Arthralgia) patients of Ayurveda hospital in Kolkata. The individual information about clinical symptoms and diagnosis by the hospital physicians were recorded.

**Biochemical Investigations**

**Determination of uric acid content in serum**

UA content in serum was determined by Kit method (Autopak, Siemens, India). It is based on the principle that UA is converted by uricase into allantoin and hydrogen peroxide which in presence of peroxidase oxidizes the chromogen to a red colored compound which is read at 500 nm with a semi automatic biochemistry analyzer (Robonik, Mumbai, India).

**Determination of Calcium content in serum**

Ca\(^{2+}\) content in serum was determined by Kit method (Autopak, Siemens, India) based upon the method of Henry and Dryer with some modifications. It is based on the principle that Ca\(^{2+}\) in an alkaline medium reacts with o-Cresolphthalein complexone to form an intense chromophore which absorbs light at 575 nm with a semi-automatic biochemistry analyzer (Robonik, Mumbai, India).

**Table 1: Incidence of age and chronicity of Sandhi shoola (Arthralgia) among patients of Ayurveda hospital**

<table>
<thead>
<tr>
<th>Age Group (years)</th>
<th>No. of patients</th>
<th>Sex</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 - 30</td>
<td>10</td>
<td></td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>30 - 45</td>
<td>16</td>
<td></td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>45 - 60</td>
<td>12</td>
<td></td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>60+ Above</td>
<td>12</td>
<td></td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td></td>
<td>27</td>
<td>23</td>
</tr>
</tbody>
</table>

* Diagnosis based upon the OPD physician of the Ayurveda hospital.

**Table 2: Biochemical Profiles of Sandhi shoola (Arthralgia) patients of Ayurveda hospital**

<table>
<thead>
<tr>
<th>Sandhi shoola Type</th>
<th>Total No. of patients</th>
<th>UA (mg/dl)</th>
<th>Ca(^{2+}) (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aamvata</td>
<td>24</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>Sandhi Vata</td>
<td>15</td>
<td>5***</td>
<td>6***</td>
</tr>
<tr>
<td>Vatarakta</td>
<td>11</td>
<td>9</td>
<td>2</td>
</tr>
</tbody>
</table>

UA-Uric acid; Ca\(^{2+}\)-Calcium, Normal range for UA - Male: 2.5 - 7.0 mg/dl, Female: 1.5 - 6.0 mg/dl, Normal range for Ca\(^{2+}\): 8.5 - 11.0 mg/dl

* P < 0.05 as compared to Aamvata group.

**Table 3: Number of patients with positive serological profiles**

<table>
<thead>
<tr>
<th>Sandhi shoola Type</th>
<th>Total No. of patients</th>
<th>RAF positive</th>
<th>CRP positive</th>
<th>ASO positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aamvata</td>
<td>24</td>
<td>12</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td>Sandhi Vata</td>
<td>15</td>
<td>5***</td>
<td>6***</td>
<td>1</td>
</tr>
<tr>
<td>Vatarakta</td>
<td>11</td>
<td>9</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

RAF:- Rheumatoid arthritis factor; CRP:- C-reactive protein; ASO:- Anti-streptolysin-O., ***P < 0.001 as compared to Aamvata group.

**Figure 1: Incidence of chronicity of Sandhi shoola (Arthralgia) among patients of Ayurveda hospital**
Serological Investigations
Qualitative determination of Rheumatoid Arthritis Factor (RAF)
RAF was determined by the latex agglutination test kit (Aspen laboratories, India). It is based on the principle that when latex reagent (polystyrene latex particles coated with human IgG) is mixed with a test serum containing RAF higher than 8 IU/ml gives visible agglutination reaction.  

Qualitative determination of C-reactive protein (CRP) in serum
C-reactive protein in serum was assessed by latex agglutination test kit (Aspen laboratories, India). CRP latex test is based on the principle that when latex reagent (polystyrene latex particles coated with anti-CRP) is mixed with a test serum containing CRP higher than 0.8 mg/dl gives visible agglutination reaction.  

Qualitative determination of Anti-streptolysin-O (ASO) antibodies in serum
An ASO antibody in human serum was determined by latex agglutination test kit (Aspen laboratories, India). CRP latex test is based on the principle that when latex reagent (polystyrene latex particles coated with streptolysin-O) is mixed with a test serum containing antibodies to streptolysin-O higher than 200 IU gives visible agglutination reaction.  

Statistical analysis
The data obtained was analyzed for significance between the groups by one-way analysis of variance (ANOVA). P value was calculated by statistical software programme “SPSS evaluation version 18”.  

RESULTS
Results showed that maximum number of Sandhi shoola (Arthralgia) patients diagnosed provisionally as common three types of Vata vyadhis were of the age group 30 to 45 years while minimum number of patients were in age group 15 to 30 (Table 1). Nearly equal numbers of male and female patients were considered in the study and they were 27 and 23 respectively. The Sandhi shoola were categorized as AV, SV and VR and results showed that maximum number of patients suffering were from AV (24) followed by SV (15) and VR (13) based on the diagnosis of the Ayurveda hospital physicians (Figure 1). Maximum numbers of patients with AV were between 30 to 60 years of age while the occurrence of SV was maximum in the patient above 60 years. There was no specific age group for the VR.

Biochemical profile of the Sandhi shoola patients
Results showed that the range of the UA and Ca$^{2+}$ were 2.6 – 18.0 mg/dl and 7.0 – 11.3 mg/dl respectively among the subjects studied (Table 2). The average UA content is maximum in case of VR, while no significant difference was found in Ca$^{2+}$ content among the three Sandhi shoola. Results indicated that there was no significant decrease in Ca$^{2+}$ content in Sandhi shoola.

Serological profile of the Sandhi shoola patients
Serological tests indicated 12 positive cases for RAF and 13 positive cases for CRP in case of AV out of total 24 patients (Table 3). Results indicated that half of the patients diagnosed with AV and SV were CRP positive in serum. However, no RAF positive agglutination reaction was observed in the patients with VR. The results showed that there were no significant numbers of patients with ASO positive titre.

DISCUSSION
In Ayurveda system of medicine subjective inferences played the vital roles in understanding of health and disease. Individualization of the treatment as per the physical and mental symptomatic characteristics of patient as well as of the diseases are often showed as the unique feature of Ayurveda and as a major principle to contemporary medicine despite of the poor reproducibility of these parameters if applied on large number of patients, creates confusion in disease understanding and treatment. Precise diagnosis based upon disease understanding also helps in analysis of the future course of the disease, the possible results of therapeutic interventions as per the current understanding of the management and prognosis of the disease. A disease pathogenesis along with the biochemical and serological evidence is essential in modern system and can surely strengthen the patient’s reliability towards the Ayurveda. Therefore, biochemical and serological diagnosis is essential to upgrade the Ayurveda practice of medicine up to the level of modern medical science. Our results showed that biochemical and serological investigations revealed a strong diagnostic measure for Sandhi shoola. According to the modern medical practice serum UA determination is an important diagnostic value in gout and kidney failure. Elevated Ca$^{2+}$ values are associated with hyperparathyroidism, multiple myeloma, neoplasias of bone and parathyroid and condition of rapid demineralization of bone, such as altered distribution of mechanical stresses, reduced area and shock-absorbing capacity of cartilage, stiffening and increased vascularization of periarticular bone and changes in musculature and weight-bearing through the joints, while lowered Ca$^{2+}$ level are associated with hyperparathyroidism, tetany, and occasionally with nephrosis and pancreatitis. The clinical significance of RA determination consists in differentiation between the rheumatoid arthritis, in which RF have been demonstrated in the serum of the approximately 80% of the cases examined and rheumatic fever in which RF are almost always absent. CRP a serum protein which is synthesized in the liver and usually appears in the sera of patients in the acute stages of the conditions like bacterial and viral infection, rheumatoid fever, rheumatoid arthritis, inflammation etc. Streptolysin-O liberated from bacteria stimulates the production of ASO antibodies in human body and detection of these antibodies is very useful for diagnosis of streptococcal infection and their relative effects such as rheumatic fever and glomerulonephritis.

Results of the present study showed that RA and CRP were positive for nearly half of the AV patients while UA
content is higher in case of VR. As RA is associated with rheumatoid arthritis, our results also emphasize on the fact that out of 24 patients of AV diagnosed, only half numbers of cases showed RA and CRP positive results. However, there is no specific biochemical and serological tests among these parameters to specify SV while radiological investigations can be more reliable in the diagnosis of SV.

CONCLUSION
Our results strongly suggested that AV can be considered similar as rheumatoid arthritis, SV as osteoarthritis and VR as gout. Therefore, the study strongly suggests that Ayurveda professionals should be encouraged for biochemical and serological investigations along with symptomatic diagnosis before reaching on conclusion about the type of Sandhi shoola. In conclusion, modern specific biochemical, molecular and serological biomarker identification for a disease should be encouraged in Ayurveda practice for diagnosis, treatment, evaluation, outcome and research.

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