Comparative analysis of the biochemical parameters among the three Prakriti individuals having Type 2 diabetes mellitus

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The occurrence of multi-organ affecting Type 2 diabetes mellitus is increasing day by day that consequently becomes a serious threat to public health. According to Ayurveda, individual’s basic constitution (Prakriti) describes its predisposition and prognosis to disease status and also the treatment for disease but no detailed studies carried out yet on diabetic individuals of three Prakriti groups on biochemical aspects. This study was conducted with twelve patients in each three Prakriti groups having Type 2 diabetes mellitus to determine the differences of some anthropometrical and biochemical parameters amongst them. Clinical profiling and various biochemical parameters of liver and kidney, lipid profiles and antioxidant enzymes were assayed. Significant differences were observed amongst the subjects belonging in different Prakriti groups. Differences in liver function enzymes and kidney function parameters and lipid profiles revealed that the subjects of Kapha and Pitta Prakriti were predominant groups. Glycosylated haemoglobin percentage was higher in Pitta Prakriti individuals. The screening process of the study indicated that diabetic individuals mostly belong to the Pitta and Kapha Prakriti groups of which Kapha Prakriti was more prone to hepatic and renal disorder and Pitta Prakriti was more susceptible towards cardiac disorder due to their diabetic complications.

Keywords: Ayurveda, Diabetes, Kapha, Pitta, Prakriti, Vata,

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In Ayurveda, Prakriti is described to be a consequence of the relative proportion of the three doshas, Vata, Pitta, and Kapha\textsuperscript{1}. An individual’s basic constitution, together these three doshas determine the physiological balance and constitution of the individual. These proportions of Tridoshas are not only genetically determined but also influenced by the environment during the development, especially maternal diet and lifestyle as described in the Shukra-Shonita\textsuperscript{2-3}. Prakriti is fixed at the time of birth and remains invariant throughout the individual’s lifespan. Others factors such as ethnicity, geography, and environment that contribute to inter individual variability at the genetic or epigenetic levels are embedded in Ayurveda’s concept of Prakriti\textsuperscript{2-3}. In an individual, the Tridoshas work in harmony and maintain homeostasis throughout the lifetime of the individual. Any alteration of an individual’s homeostatic state of Dosha can leads to various diseases. Modern science of genetics has established the fact that the individual of different constitutional types expresses different set of genes that governs a specific biological process\textsuperscript{4}.

Type 2 diabetes mellitus is a predictable and preventable disease. Obesity, familial history, and higher glucose levels (> 150 mg/dL) are well-known risk factors for development of Type 2 diabetes in the middle age. It has been estimated that there are 451 million (age 18–99 years) people with diabetes worldwide in 2017 which are expected to increase to 693 million by 2045 as mentioned in the recent report of International diabetic Federation\textsuperscript{5}.

Recent prediction models have incorporated these with other readily measurable features of metabolic syndrome such as elevated blood pressure, low HDL cholesterol and elevated triglycerides to generate validated prediction rules. Non-alcoholic fatty liver disease (NAFLD) is associated with Type 2 diabetes and the metabolic syndrome, which further aggravates the progress to chronic liver disease\textsuperscript{6}. On the other hand renal disease in Type 2 diabetes is more heterogeneous than in Type 1 diabetes mellitus\textsuperscript{7}.
Serum creatinine and urea concentration are widely used as an index of renal function. Elevated levels of serum creatinine and creatinuria may be observed in extensive muscle destruction. Amino acids and triglycerides are the signature markers to predict increased risk of Type 2 diabetes. Patients with Type 2 diabetes are at increased risk of cardiovascular disease, partly owing to dyslipidaemia, which can be amenable to fibrate therapy. Insulin resistance is the best prediction factor for the clinical onset of Type 2 diabetes. Intramuscular triglycerides storage may be a primary pathogenic factor for its development. People with diabetes generally have lower HDL-Cholesterol and higher triglycerides concentration. The present study was focused on a comparative analytical study of the different biochemical parameters among the diabetic individuals who were belonging in the different Prakriti groups as referred by ancient Ayurvedic literature.

Methodology

Glycosylated haemoglobin kit was provided by Biosystems, Barcelona, Spain. Acid phosphatase (ACP) kit was purchased from Accurex Biomedical Pvt. Ltd., Mumbai, India. Urea, Alkaline phosphatase (ALP), Serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvate transaminase (SGPT) kits were supplied by Piramal Healthcare Limited, Mumbai, India. Total cholesterol kit was supplied by Beacon Diagnostic Pvt. Ltd. Triglyceride and creatinine was measured by using the kits provided by Merckotest®, Merck, Goa, India. All the biochemical tests were repeated twice.

First, the ethical clearance was obtained from the Institutional Ethical Committee of J B Roy State Ayurvedic Medical College and Hospital, Kolkata (JBR/IEC/05/10/2012). The participants have been included in the study after receiving their duly signed consent form. The blood samples have been collected specially from the outdoor patients of the J B Roy State Ayurvedic Medical College and Hospitals, Kolkata with the help of authorized medical practitioners. The patients were of both sexes and aged between 30-70 years. All the participants were given detailed information and explanation about this study.

Identification of Prakriti phenotypes

The subjects were screened through the short Prakriti questionnaire specific for Type 2 diabetes mellitus, developed in accordance with the guideline of the CSIR-TRISUTRA (Table 1A - C) research project. The questionnaire was based on individual’s personal background such as; ethnicity, linguistic lineage etc. as well as familiar history of disease. It also involved some personal diet and life style related questions including allergies to food or drugs etc. A detailed history of clinical information including age, sex and recurrent illness etc. was taken.

In the second part, the main emphasis was on analysis of Dosha Prakriti of an individual through general examination and questioning. This part involved certain points which might be subjected to variation owing to one’s lifestyle, personal habits, certain recent changes in diet, sleep and life style etc. These were designed carefully on the basis of their personal liking and lifestyle. After proper screening, the Prakriti determination was done by the analysis of the short questionnaire. Then the participant was subjected to detailed objective phenotyping where height, weight, body mass index (BMI) etc. were measured. Glycemic status was assessed using fasting and two hours postprandial blood glucose levels.

Sample processing for biochemical analysis

Blood samples were collected in a heparinized tubes or vaccutainers containing ethylenediamine tetraacetic acid di-sodium salt (EDTA-2Na) and also in a plain tube or without EDTA coated vaccutainers. Simultaneously plasma, serum and erythrocytes were separated immediately after collection through the centrifugation at $1,500 \times g$ for 10 min at $4^\circ C$. The plasma and serum were subsequently frozen and stored at $-80^\circ C$ for further analysis.

Biochemical analysis

Glycosylated haemoglobin was assessed from whole blood by using the kit as provided by Biosystems. Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) in human serum was assayed by Reitman and Frankel Method as demonstrated in the kit. Serum Alkaline Phosphatase level was measured by Kind and king’s method as described in the kit.

Serum urea concentration was detected by Diacetylmonoxime (DAM) method as instructed in the kit. In this method the urea from the blood serum reacted with hot acidic Diacetylmonoxime in presence of Thiosemicarbazide and produced rose purple colour complex which is measured by UV-Vis spectrophotometer. Serum Creatinine level was measured according to the method described in the kit.
The method is known as Alkaline Picrate Method where creatinine in a protein free solution reacts with Alkaline Picrate and produced a red coloured complex which was measured spectrophotometrically.

The total cholesterol, HDL cholesterol and LDL cholesterol in serum were measured according to the method of Warnick et al., supplied in the kit. The triglycerides were measured according to the method of Stein et al.

Statistical analysis

The data are presented with mean + S.E. (n = 12 in each group). The significance levels of each determined parameter were done by using one way ANOVA.
Results

Three Prakriti groups each comprising 12 individuals i.e., total 36 diabetic individuals were subjected for the study. Among those individuals, 73% were males and 27% were females. The age of the patients was ranged between 30-70 years. Among the three groups of Prakriti individual the mean age of Vata group was 49 years and Pitta and Kapha groups were 53 years respectively. The mean height and weight of Vata group were 159.20 cm and 61.91 kg respectively with BMI of 22.33 kg/m², and that of Pitta group were 161.75 cm and 64.66 kg respectively with BMI of 22.41 kg/m² and Kapha group were 162.37 cm and 66.16 kg respectively with BMI of 25.67 kg/m². The mean waist and hip circumferences of Vata were 89.72 cm and 90.15 cm, Pitta 89.80 cm and 90.66 cm and Kapha 94.59 cm and 93.90 cm respectively. The mean random blood glucose level of the Vata group was 238.32 mg/dL, Pitta and Kapha were 242.06 mg/dL and 206.44 mg/dL respectively. The clinical parameters according to the Prakriti distribution are shown in Fig. 1.

Biochemical analysis of this study showed that the mean value of glycosylated haemoglobin was higher (> 7) for all the individuals. The mean value of glycosylated haemoglobin in Pitta group (10.04±0.68) was found to be higher than that of other Prakriti groups. The elevated level of hepatic enzymes was observed in Kapha Prakriti individual. They contain higher level of alanine aminotransferase (50.97±10.85), and alkaline phosphatase (23.37±2.64). Whereas the higher level of aspartate aminotransferase enzyme (20.52±6.76) and acid phosphatase ((1.13±0.30) were observed in the Vata Prakriti individual. The level of alkaline phosphatase enzyme was varied significantly (p < 0.05) between the three Prakriti groups of individual.

The mean value of urea for Kapha group (23.86±1.87) was slightly elevated than the Vata group (21.45±2.51) and significantly greater than of the Pitta group (16.50±2.06) whereas the mean creatinine value of Kapha group (1.07±0.55) was almost double that of the Vata (0.55±0.07) and Pitta (0.59±0.05) groups.

The total cholesterol, and low density lipoprotein were varied significantly between the three Prakriti types of individual. The mean LDL level and total cholesterol were also higher in the Pitta Prakriti (133.83±9.98 and 211.95±9.64 respectively) in comparison with Vata (94.16±7.12 and 164.19±9.18.

![Fig. 1 — Graphical representation of comparative study of physical parameters including Height, Weight, BMI, Hip circumferences and Waist circumferences and Random blood glucose between the three group of Prakriti individual. Data are presented as Mean ± S.E, (n=12). Significance levels are determined using one way ANOVA. The bar graph (Fig. A) shows the mean height of the three contrasting Prakriti individual where Kapha Prakriti showing the maximum mean height. The Fig. B shows the mean weight and Fig. C shows the mean BMI of the diabetic individual belongs to three Prakriti Type. Here also Kapha Prakriti showing the maximum weight and highest BMI. Fig. D, E, F represents the comparative data of Hip circumferences, Waist circumferences and Random Blood Glucose respectively. Among the three Prakriti groups of diabetic individual Kapha Prakriti shows the highest value of Hip and waist circumferences and Pitta Prakriti shows the highest level of random blood glucose.](image-url)
respectively) and Kapha (96.75±6.67 and 180.16±11.27 respectively) Prakriti. Whereas the mean HDL value was higher in the Vata Prakriti group (44.00±2.15) compared to Pitta (40.75±2.28) and Kapha (39.40±1.87) Prakriti groups. High level of very low density lipoprotein was found in Kapha (43.91±5.52) Prakriti group in comparison to the Vata (26.03±3.02) and Pitta (37.36±4.80) Prakriti groups.

The significance levels of the each parameter as determined in this study were tabulated in Tables 2 and 3.

**Discussion**

Ayurveda is the ancient health science of the world, practiced since 1500 BC Ayurveda deals with body, mind and spirit which were based on the concept of Tridoshas and Prakriti. Prakriti means manifestation of special characteristics due to predominance of Doshas (Vata, Pitta and Kapha the functional component of body). All the physiological process of the body is directly under the regulation of these Tridoshas; Vata, Pitta and Kapha (three physical humors). The clinical manifestations of various diseases are specific to the type of Prakriti that is suffering from the disease. Healthy status can be maintained by maintaining the normality of Prakriti which determines the strength of a person to combat with the diseases. Equilibrium of Prakriti in an individual is said to be having best immunity and strength. Any imbalance of the homeostatic phases of doshas is called diseases or Vikriti (Ashtanga Hridaya, Sutra Sthana 1). Disequilibrium in Prakriti greatly increases the risk of diseases16-17.

The screening process of the diabetic patients showed that among the three groups of diabetic individuals most of the patients (n = 75) were belong to the Pitta and Kapha Prakriti group (unpublished data). This study comprised of 12 patients in each Prakriti group i.e., total 36 Type 2 diabetes mellitus patients were chosen arbitrarily and their different biochemical parameters were determined and compared. The mean glycosylated haemoglobin percentage in Pitta group (10.04±0.68) was higher in comparison than that of Vata (9.70±0.72) and Kapha (8.68±0.36) which suggests that the individual belonging to Pitta Prakriti group is more prone towards diabetes (Fig. 2). Though the mean percentage of glycosylated haemoglobin of Vata Prakriti was comparatively higher than that of Kapha Prakriti, but the total number of diabetes affected individuals was more in Kapha Prakriti than Vata Prakriti as observed during screening process. This may be the fact that the diabetic individuals belonging to Vata Prakriti or Vata predominant Kapha Prakriti (i.e., mixed type or Dwanja Prakriti) are severely affected by the disease and have more complications due to the onset of the disease18.

Diabetes caused dysfunction as well as damage of the liver cells19, due to which the liver function enzymes ALT and AST levels were increased.

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**Table 2 — Significance level of physical parameters among the three predominant groups of Prakriti individual having type 2 Diabetes mellitus**

<table>
<thead>
<tr>
<th>Physical Parameters</th>
<th>P- Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (cm)</td>
<td>0.628</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>0.629</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.298</td>
</tr>
<tr>
<td>Hip Circumferences (cm)</td>
<td>0.298</td>
</tr>
<tr>
<td>Waist Circumferences (cm)</td>
<td>0.233</td>
</tr>
<tr>
<td>Random Blood Sugar (mg/dL)</td>
<td>0.579</td>
</tr>
</tbody>
</table>

**Table 3 — Significance level of the biochemical parameters among the three predominant groups of Prakriti individual having type 2 Diabetes mellitus**

<table>
<thead>
<tr>
<th>Biochemical Parameters</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1c (%)</td>
<td>0.279</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>0.138</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>0.452</td>
</tr>
<tr>
<td>Alkaline Phosphatase (KA Unit)</td>
<td>0.000</td>
</tr>
<tr>
<td>Acid Phosphatase (Unit/mL)</td>
<td>0.428</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>0.095</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.473</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dL)</td>
<td>0.007</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>0.002</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>0.311</td>
</tr>
<tr>
<td>VLDL (mg/dL)</td>
<td>0.030</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>0.030</td>
</tr>
</tbody>
</table>

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**Fig. 2 — Graphical representation of comparative study of glycosylated haemoglobin (HbA1c) levels between the three groups of Prakriti individual. Data are presented as Mean ± S.E, (n = 12). Significance levels are determined using one way ANOVA. The diabetic individual having Pitta Prakriti shows the highest percentage of glycosylated haemoglobin than the other two Prakriti groups.**
Elevated hepatic enzymes, alanine aminotransferase [ALT], aspartate aminotransferase [AST] are associated with prospective changes in liver or whole-body insulin sensitivity and or insulin secretion. Alkaline phosphatase is another serum marker widely used to detect the myocardial infarction and bone disorder. This enzyme is present in many human tissues, including bone, intestine, kidney, liver, placenta and white blood cells. Damage to these tissues causes the release of alkaline phosphatase (ALP) into the bloodstream. Elevated alkaline phosphate is also associated with Type 2 Diabetes Mellitus. Comparatively higher level of ALT (50.97±10.85) was observed in Kapha Prakriti than Vata and Pitta Prakriti (Fig. 3). The levels of AST were higher in Vata and Kapha Prakriti than Pitta Prakriti as observed in Fig. 3. Similarly, the activity of ALP was more in Kapha (23.37±2.64) and Pitta (22.66±3.35) Prakriti than Vata Prakriti. Comparatively, higher level of ACP activity (1.13±0.08) was observed in the Vata Prakriti individual. The levels most of the liver function enzymes were found to comparatively be lower in Pitta Prakriti which indicates that the diabetic patients belonging to Pitta Prakriti suffer from comparatively less hepatic complications than other Prakriti diabetic patients.

The mean urea value of Kapha group was slightly elevated than the Vata group and much greater than the Pitta group whereas the mean creatinine value of Kapha was largely elevated than the other two groups as seen from Fig. 4. This implies that the individuals belonging to Kapha Prakriti are more susceptible to renal disorders than the two other groups.

Diabetes also causes disorder in carbohydrate
metabolism that leads to disorder in lipid metabolism because carbohydrates and lipid metabolism are interrelated to each other. Diabetes thus increased the concentrations of cholesterol and triglycerides levels within the blood. The total cholesterol, and low density lipoprotein were varied significantly between all the three Prakriti types of individuals. The concentrations of total cholesterol and LDL-cholesterol were comparatively higher in Pitta Prakriti diabetic individuals (Fig. 5). Interestingly, the concentration of HDL was comparatively lower and VLDL as well as triglycerides levels were comparatively higher as determined in Pitta Prakriti diabetic individuals. The lipid profiles of Vata Prakriti were comparatively better than the other two groups. Our observations thus indicate that the diabetic patients belonging to Pitta Prakriti are more susceptible to cardiac disorders whereas the diabetic individuals belonging to Vata Prakriti are comparatively in safe zone towards cardiac problems.

Fig. 4 — Graphical representation of comparative study of the renal function including Urea (Fig. A) and Creatinine (Fig. B) concentrations in serum (mg/dL) between the three groups of Prakriti individual. Data are presented as Mean ± S.E, (n=12). Significance levels are determined using one way ANOVA. Fig. A and B shows the presence of elevated level of urea and creatinine in the serum of the Kapha Prakriti having Type 2 diabetes mellitus.

Fig. 5 — Graphical representation of comparative study of the lipid profiles including total cholesterol (Fig. A), LDL (Fig. B), HDL (Fig. C),VLDL (Fig. D), triglycerides (Fig. E) concentration in serum (mg/dL) between the three groups of Prakriti individual. Data are presented as Mean ± S.E (n=12). Significance levels are determined using one way ANOVA. Fig. A and B shows the presence of increased level of total cholesterol and Low density lipoprotein (LDL) in the serum of the diabetic Pitta Prakriti and the elevated concentration of high density lipoprotein (HDL) in the serum of diabetic Vata Prakriti. Fig. D and F indicates the presence of higher concentration of Very low density lipoprotein (VLDL) and triglycerides in diabetic Kapha Prakriti.
Conclusion
In the present study, the screening process showed that among the three groups of diabetic individuals most of the patients were belonging to the Pitta and Kapha Prakriti groups. A striking difference in the renal enzymes, hepatic enzymes and lipid profile were observed between the three groups of individual. Kapha and Pitta group were found to be the most susceptible group having more complications associated with Type 2 diabetes mellitus. The results showed that Pitta Prakriti was comparatively safe group towards hepatic disorder whereas, the Kapha group was the more prone towards the renal disorder as well as hepatic disorder. Vata Prakriti was the comparatively at safe zone towards cardiac disorder as they had higher level of good cholesterol i.e., HDL and comparatively lower level of the other bad cholesterol and triglycerides.

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Conflict of Interest: There is no conflict of interest of any kind.

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