Serum Inorganic Phosphate Concentration and Glycated Haemoglobin Percent in Type 2 Diabetes Mellitus - A Hospital Based Study

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ABSTRACT

Objective:
1. To study serum inorganic phosphate concentration and glycated hemoglobin percent in patients with type 2 diabetes mellitus.
2. To find the correlation between serum inorganic phosphate level and glycated hemoglobin level in patients with type 2 diabetes mellitus.

Materials and methods: The study was carried out on 50 newly diagnosed patients of type 2 diabetes and controls in the Department of Biochemistry, Assam medical college, Dibrugarh. Analysis of blood glucose (fasting and postprandial), HbA1c, serum inorganic phosphate, urea and creatinine concentrations were performed by standard methods using semi automated analyser.

Results: Concentrations of fasting and postprandial blood glucose higher in the diabetic group than controls (p<0.01), and the mean HbA1c% was also higher in cases (8.82±1.66%). The mean serum inorganic phosphate concentration in cases was found to be significantly lower than controls (2.68±0.56 vs 3.64±0.42 mg/dl) and p < 0.01. Present study revealed an inverse relationship between serum inorganic phosphate concentration and HbA1c% in patients with type 2 DM with a correlation coefficient, r = -0.81 substantiated by regression analysis.

Conclusion: There is definite reduction of serum inorganic phosphate concentration in type 2 DM patients and that reduction of serum inorganic phosphate concentration may have a contributing role in the progression of the disease and development of complications of diabetes.

Keywords: diabetes, inorganic phosphate, glycated hemoglobin.

INTRODUCTION

Diabetes mellitus is a group of metabolic disorders of carbohydrate metabolism in which glucose is underused, producing hyperglycemia. [1] There are two main forms of diabetes. Type 1 diabetes is due primarily to autoimmune-mediated destruction of pancreatic b-cell islets, resulting in absolute insulin deficiency. Its frequency is low relative to type 2 diabetes, which accounts for over 90% of cases globally. Type 2 diabetes is characterized by insulin resistance and/or abnormal insulin secretion, either of which may predominate. [2]

Diabetes is an “iceberg” disease. Although increase in the prevalence and incidence of type 2 diabetes have occurred globally, they have been especially dramatic in societies in economic transition, in newly industrialized countries and in developing countries. Currently the number of cases of
diabetes worldwide is estimated to be around 347 million; of these more than 90 percent are type 2 diabetes. [3]

Diabetes is a major cause of mortality, but several studies indicate that diabetes is likely underreported as a cause of death. A recent estimate suggested that diabetes was the fifth leading cause of death worldwide and was responsible for almost 4 million deaths in 2010 (6.8% of deaths were attributed to diabetes worldwide). [4]

Type 2 diabetes is a global public health crisis that threatens the economies of all nations, particularly developing countries. Fueled by rapid urbanization, nutrition transition, and increasingly sedentary lifestyles, the epidemic has grown in parallel with the worldwide rise in obesity. Asia’s large population and rapid economic development have made it an epicenter of the epidemic. [5]

The recently published ICMR-INDIAB national study reported that there are 62.4 million people with type 2 diabetes (T2DM) and 77 million people with prediabetes in India. These numbers are projected to increase to 101 million by the year 2030. The complications related to diabetes pose a significant health care burden and a deterrent to overall quality of life. The Chennai Urban Population Study (CUPS) and Chennai Urban Rural Epidemiology Study (CURES) are one of the few population based studies on complications of diabetes in India and show that there is a huge burden due to diabetes related complications in India. The prevalence of diabetic retinopathy (DR) was 17.6%, microalbuminuria was 26.9%, neuropathy was 26.1%, coronary artery disease (CAD) was 21.4% and peripheral vascular disease (PVD) was 6.3%. The cost of treatment for diabetic complications adds to the health care costs. India thus faces a huge health care burden due to high prevalence of type 2 diabetes and its complications. [6]

An adult has about 600g or approximately 20 mol of phosphorus as inorganic and organic phosphates, of which about 85% is in the skeleton, and the rest is principally in soft tissue. Plasma contains both inorganic and organic phosphate, but only inorganic phosphate is measured. Inorganic phosphate is a major component of hydroxypapatite in bone; thus it plays an important role in the structural support of the body and provides phosphate for the extracellular and intracellular pool. [7]

Early in the progression of diabetes, a paradoxical metabolic imbalance in inorganic phosphate (Pi) occurs that may lead to reduced high energy phosphate and tissue hypoxia. These changes take place in the cells and tissues in which the entry of glucose is not controlled by insulin, particularly in poorly regulated diabetes patients in whom long-term vascular complications are more likely. Various conditions are involved in this disturbance in Pi. First, the homeostatic function of the kidneys is suboptimal in diabetes, because elevated blood glucose concentrations depolarize the brush border membrane for Pi reabsorption and lead to lack of intracellular phosphate and hyperphosphaturia. Second, during hyperglycemic-hyperinsulinemic intervals, high amounts of glucose enter muscle and fat tissues, which are insulin sensitive. Intracellular glucose is metabolized by phosphorylation, which leads to a reduction in plasma Pi, and subsequent deleterious effects on glucose metabolism in insulin insensitive tissues. While low and high uncontrolled blood sugars give rise to easily recognizable clinical symptoms, low and high plasma inorganic phosphate remains unrecognizable or presents vague and general symptoms. Hypophosphatemia is strongly related to a decrease in intracellular adenosine triphosphate (ATP) in the aging process and in uremia. Any interruption of optimal ATP production might lead to cell injury and possible cell death. [8]

In the past several decades, detection of glycated hemoglobin (HbA1c) has become a standard in diabetic patient care. With the increasing understanding of the role of HbA1c in diabetes management,
HbA\textsubscript{1c} has become one of the hotspots in clinical research today. HbA\textsubscript{1c} reflects the average blood sugar level of patients over a period of time, and large-scale studies have shown that HbA\textsubscript{1c} is a test index with the highest correlation with diabetic retinopathy, diabetic nephropathy and diabetes neuropathy. In addition, HbA\textsubscript{1c} is more stable and affected by fewer factors than fasting blood sugar and 2-hour postprandial blood sugar and is becoming increasingly used in clinical applications. HbA\textsubscript{1c} is not only used as a monitoring index to control blood sugar in patients with diabetes, but is also used for screening and diagnosis of diabetes. The combinations of HbA\textsubscript{1c}, blood glucose point values and dynamic blood sugar monitoring provides health care providers with a better understanding of the patient’s ability to control his or her blood sugar level, as well as a means to monitor and predict the risks associated with chronic diabetes.\textsuperscript{[9]}

In this background, it has become necessary to study the status of serum inorganic phosphate in type 2 diabetes mellitus and its association with glycated hemoglobin in this region where no such study has been undertaken before. Therefore a humble effort is made to undertake this study with the following aims and objectives.

**MATERIAL AND METHODS**

The present study “serum inorganic phosphate concentration and glycated hemoglobin percent in type 2 Diabetes Mellitus” is a hospital based study carried out on diagnosed cases of type 2 diabetes mellitus that attended and/or was admitted in the Department of Medicine, Assam Medical College and Hospital. The study was conducted in the Department of Biochemistry and Advanced Clinical Biochemistry Laboratory, Assam Medical College and Hospital, Dibrugarh.

**Place of study** : Department of Biochemistry, Advanced Clinical Biochemistry Laboratory, Assam Medical College and Hospital.

**Duration of study** : August’ 2013 to September’ 2014

**No of cases** : 50 (fifty)

**No of controls** : 50 (fifty)

**Type of study** : Case-Control Study.

**Selection of cases** : Patients were selected randomly on a weekly basis.

**Inclusion criteria**:
- Diagnosed cases of type 2 diabetes mellitus that attended and/or was admitted in Department of Medicine, Assam Medical College and Hospital.
- Patients were selected on the basis of clinical history, clinical examination and relevant clinical investigations. The individuals were selected irrespective of sex and socio-economic status. Sex - Male and Female.
- Controls - age and sex matched healthy subjects.

**Exclusion criteria:**
- Patients with diagnosed type 1 diabetes mellitus.
- Patients who did not give consent to the study.
- Pregnant women.
- Patients receiving Phosphate supplementation.
- Patients with Chronic Renal Diseases.
- Patients on drugs that alter serum phosphate level or drugs modifying metabolism of phosphate.
- Patients with hepatic disorders, renal disorders, malignancies, myocardial infarction, patients on diuretics, bleeding disorders and other major illness.
- Smokers and Alcoholics.

**Criteria for diagnosis of diabetes mellitus**

Patients will be considered diabetic according to the criteria defined by American Diabetes Association 2011 criteria.\textsuperscript{[10]}

For all parameters, a semi-autoanalyzer (MERCK Microlab 300) was used. Required reagents were used for estimation of parameters.

**Test procedures**

- Serum inorganic phosphate (molybdate u.v. method)
Glycated hemoglobin (Method: Ion exchange resin)

**Statistical analysis**

Arithmetic mean and standard deviation were worked out to assess the levels of various parameters in both groups under study. Students’ t test was used for comparison of quantitative variables. Co-relation between serum inorganic phosphate and HbA1c% in patients was evaluated using Pearson Co-relation Co-efficient. All tests were considered statistically significant if the p-value was <0.05. All statistical analysis was done in Microsoft Excel, Graphpad.

**RESULTS**

Table 1: showing range and mean ± SD of serum inorganic phosphate in study subjects

<table>
<thead>
<tr>
<th>Study Group</th>
<th>Serum Inorganic Phosphate (Mg/Dl)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>CASES</td>
<td>1.9 ± 4.0</td>
<td>2.73 ± 0.58</td>
</tr>
<tr>
<td>CONTROLS</td>
<td>2.8 ± 4.4</td>
<td>3.64 ± 0.46</td>
</tr>
</tbody>
</table>

It is observed from table 1 that the mean serum inorganic phosphate level in male cases had been 2.73 ± 0.58 mg/dL and that in female cases 2.6 ± 0.54 mg/dL. In the control group, the mean serum inorganic phosphate level in males had been 3.64 ± 0.46 mg/dL and that in female, 3.63 ± 0.36 mg/dL. The difference between the mean serum inorganic phosphate level between males and females in cases and in controls was very minimal and it was statistically not significant (p>0.05).

Table 2: Age-wise distribution of mean serum inorganic phosphate levels in study subjects

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Serum Inorganic Phosphate (Mg/Dl)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>&lt;40</td>
<td>1.9 – 3.1</td>
<td>2.35 ± 0.48</td>
</tr>
<tr>
<td>41-50</td>
<td>2.0 – 4.0</td>
<td>2.85 ± 0.55</td>
</tr>
<tr>
<td>51-60</td>
<td>2.0 – 3.7</td>
<td>2.81 ± 0.60</td>
</tr>
<tr>
<td>61-70</td>
<td>1.9 – 3.2</td>
<td>2.51 ± 0.51</td>
</tr>
<tr>
<td>&gt;70</td>
<td>2.0 – 2.9</td>
<td>2.40 ± 0.42</td>
</tr>
<tr>
<td>Total</td>
<td>1.9 – 4.0</td>
<td>2.68 ± 0.56</td>
</tr>
</tbody>
</table>

Overall, the mean serum inorganic phosphate level in cases was 2.68 ± 0.56 mg/dL and that in controls is 3.64± 0.42 mg/dL. The difference between the mean serum inorganic phosphate level between males and females in cases and in controls was very minimal and it was statistically not significant (p>0.05).

Table 3: showing mean, range and mean HbA1c% level in study subjects

<table>
<thead>
<tr>
<th>STUDY GROUP</th>
<th>HbA1c %</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>CASES</td>
<td>6.47-11.84</td>
<td>8.63 ±1.55</td>
</tr>
<tr>
<td>CONTROLS</td>
<td>3.96-5.89</td>
<td>4.95 ±0.49</td>
</tr>
</tbody>
</table>

It is observed from Table 3, that in male cases the mean HbA1c% was found to be 8.63 ± 1.55% and that in females, it was 9.09 ± 1.82%. In the control group it is seen
that the mean HbA$_{1c}$% was 4.95 ± 0.49% in males, and that in females it is 5.01 ± 0.56%. The difference in the mean HbA$_{1c}$% between males and females in cases as well as in controls was minimal and it was statistically not significant (p>0.05).

Overall, the mean HbA$_{1c}$% in cases was 8.82 ± 1.66% and that in controls was 4.97 ± 0.52%. It is seen that the HbA$_{1c}$% level in cases was higher than that in controls, and was statistically highly significant (p<0.01).

Table 4: Age-wise distribution of mean HbA$_{1c}$ % in study subjects:

<table>
<thead>
<tr>
<th>Age Group</th>
<th>HbA$_{1c}$ %</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case</td>
<td>Control</td>
</tr>
<tr>
<td>Range</td>
<td>Mean ± SD</td>
<td>Range</td>
</tr>
<tr>
<td>&lt;40</td>
<td>7.73-11.84</td>
<td>9.74 ± 1.58</td>
</tr>
<tr>
<td>41-50</td>
<td>6.47-11.59</td>
<td>8.30 ± 1.47</td>
</tr>
<tr>
<td>51-60</td>
<td>6.47-12.17</td>
<td>8.42 ± 1.72</td>
</tr>
<tr>
<td>61-70</td>
<td>7.23-12.93</td>
<td>9.59 ± 1.85</td>
</tr>
<tr>
<td>&gt;70</td>
<td>7.40-11.08</td>
<td>9.30 ± 1.51</td>
</tr>
<tr>
<td>Total</td>
<td>6.47-12.93</td>
<td>8.82 ± 1.66</td>
</tr>
</tbody>
</table>

Table 5: showing the mean and standard deviation of serum inorganic phosphate in different levels of HbA$_{1c}$ %.

<table>
<thead>
<tr>
<th>HbA$_{1c}$ Range</th>
<th>No.</th>
<th>Mean ± SD</th>
<th>HbA$_{1c}$ %</th>
<th>Inorganic phosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.00-7.50</td>
<td>14</td>
<td>6.99 ± 0.35</td>
<td>3.26 ± 0.45</td>
<td></td>
</tr>
<tr>
<td>7.51-9.00</td>
<td>15</td>
<td>8.21 ± 0.43</td>
<td>2.78 ± 0.35</td>
<td></td>
</tr>
<tr>
<td>9.01-10.50</td>
<td>11</td>
<td>9.68 ± 0.32</td>
<td>2.35 ± 0.28</td>
<td></td>
</tr>
<tr>
<td>≥ 10.51</td>
<td>10</td>
<td>11.37 ± 0.76</td>
<td>2.06 ±0.15</td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>50</td>
<td>8.82 ± 1.66</td>
<td>2.68 ± 0.56</td>
<td></td>
</tr>
</tbody>
</table>

It is observed from the table 5 that cases in the HbA$_{1c}$ range of 6.00 – 7.50 % have highest concentration of serum inorganic phosphate i.e. 3.26 ± 0.45 mg/dL. On the contrary, the HbA$_{1c}$ range of 10.51 % and above have lowest concentration of serum inorganic phosphate i.e.2.06 ± 0.15 mg/dL. So, the cases having lower values of HbA$_{1c}$ have higher values of serum inorganic phosphate concentration and vice versa.

It is seen from table 6 that serum inorganic phosphate levels showed a negative correlation with HbA$_{1c}$%, which was found to be statistically significant (p<0.01). The Pearson correlation coefficient “r” which was found to be -0.81 established the strong negative correlation between the two parameters.

Table 6: showing the correlation between glycated hemoglobin (HbA$_{1c}$ %) and serum inorganic phosphate levels in diabetic cases.

<table>
<thead>
<tr>
<th>HbA$_{1c}$ Range</th>
<th>Serum Inorganic phosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r - value</td>
</tr>
<tr>
<td>6.47-12.93</td>
<td>-0.81</td>
</tr>
</tbody>
</table>

Fig 1: showing the correlation between glycated hemoglobin (HbA$_{1c}$ %) and serum inorganic phosphate in diabetic cases.
Regression analysis:
In the equation $y = a + bx$
Taking $y = \text{serum inorganic phosphate level}$ and $x = \text{HbA1c\%}$
Regression analysis revealed that $a= 5.0958$ and $b= (-0.2737)$
Now, the equation becomes,
Serum inorganic phosphate level = $5.0958 + (-0.2737) \times \text{HbA1c\%}$
Thus, the serum inorganic phosphate level can be calculated from the HbA1c\%.

DISCUSSION
Diabetes mellitus (DM) refers to a group of common metabolic disorders that share the phenotype of hyperglycemia. Several distinct types of DM are caused by a complex interaction of genetics and environmental factors. Depending on the etiology of the DM, factors contributing to hyperglycemia include reduced insulin secretion, decreased glucose utilization, and increased glucose production. The present study was undertaken in an attempt to understand the role of serum inorganic phosphate in diabetes mellitus as well as to understand the interplay between serum inorganic phosphate level and glycated hemoglobin percentage in the progression of type 2 diabetes mellitus.

In the present study it was observed that the mean serum inorganic phosphate level in male with type 2 diabetes was $2.73 \pm 0.58 \text{ mg/dL}$ and those in female cases, $2.6 \pm 0.54 \text{ mg/dL}$. Here, the difference between male and female cases was very minimal and was statistically not significant ($p > 0.05$).

In control group also, the difference between male and female cases was very minimal and it was statistically not significant ($p > 0.05$). The above findings indicate the serum inorganic phosphate levels are independent of the gender of the study groups. Haap M et al \cite{11} in 2006 showed that there was a significant association of low serum phosphate concentration with high 2-h blood glucose levels independent of anthropometric parameters like body fat, age and gender.

In the present study, the mean level of serum inorganic phosphate in diabetic cases was found to be $2.68 \pm 0.56 \text{ mg/dL}$. On the contrary, in the control group the mean serum inorganic phosphate level was found to be $3.64 \pm 0.42 \text{ mg/dL}$. The serum inorganic phosphate levels in diabetic cases was found to be lower than that in the control group and it was statistically highly significant ($p < 0.01$).

The findings of the present study are consistent with findings of Ugwuja EI et al \cite{12} in 2007. They had examined serum phosphate level along with other parameters in 60 Diabetic patients and compared with 60 apparently healthy, age and sex matched controls. They found that serum phosphate levels are significantly lower in diabetic patients than in controls ($p < 0.05$). In diabetes mellitus, increased urinary loss due to osmotic diuresis may be the common and most important cause of reduced phosphate, although intracellular shift may also be a factor.

Osmotic diuresis present in hyperglycemic and acidemic states may cause an increased phosphate excretion due to competition between phosphate and glucose in proximal tubular system. \cite{11}

In Diabetes Mellitus, a major disturbance in phosphate handling occurs in the kidney tubules, where the excessive sodium-dependent glucose reabsorption depolarizes the electrochemical sodium gradient. Since inorganic phosphate use the same driving force, but have less binding to sodium than glucose, the inorganic phosphate reabsorption, particularly in poorly regulated patients, becomes impaired. This paradoxical phosphate imbalance may lead to affinity hypoxia and impaired formation of high energy phosphates. The lack of intracellular phosphate complementary to the increased intracellular glucose takes place in the insulin-insensitive cells and tissues, resulting in the possibility of late complications of diabetes mellitus. \cite{13}

The findings of present study are also supported by study of Nagasaka S et
In NIDDM (Type 2 DM), abnormally high blood glucose is linked to non physiologic variations in plasma insulin content throughout a 24-hr period. During hyperglycemic-hyperinsulinenic conditions, high amounts of glucose enter in to muscle and fat tissues which are insulin sensitive. Intra cellular glucose is metabolized by phosphorylation which leads to lower levels of plasma phosphate and consequent negative effects on glucose metabolism in the insulin-insensitive tissues. [8]

Ditzel et al [15] studied renal handling of Pi (inorganic phosphate) in 26 conventionally treated diabetic children and in 28 healthy children and found fasting urinary phosphate excretion 3 times higher in the former group despite a significantly lower fasting Pi. The maximal capacity of renal tubular reabsorption of phosphate per liter of filtrate (TmPO4/GFR) was significantly suppressed in the diabetic patients. The increased urinary phosphate excretion correlated positively with both urinary glucose excretion and blood glucose concentration (P <0.01). This finding was unrelated to serum PTH or to plasma growth hormone. [15]

Kalaitzidis R et al [16] in 2005 observed that patients with metabolic syndrome showed significantly lower phosphate and magnesium levels compared with controls. The findings in the present study were consistent with the reports of the above workers.

It was observed from the present study that cases in the HbA1c range of 6.00-7.50 % had highest concentration of serum inorganic phosphate i.e. 3.26 ± 0.45 mg/dL. On the contrary, the HbA1c range of 10.51 % and above had lowest concentration of serum inorganic phosphate i.e.2.06 ± 0.15 mg/dL. So, the cases having higher values of HbA1c have lower values of serum inorganic phosphate concentration and vice versa.

It was also observed in the present study that serum inorganic phosphate levels showed a negative correlation with HbA1c % that was statistically highly significant (p<0.01). The Pearson correlation coefficient “r” found to be -0.81 established the strong negative correlation between the two parameters.

The findings of present study are almost consistent with findings of Nagasaka S et al [14] in 1995. They showed that in NIDDM patients at the time of admission in to hospital, HbA1c was 11.1 ± 0.3% and the serum phosphate was 1.12 ± 0.03 mmol/l. The values of HbA1c and serum phosphate became 9.3 ± 0.2% and 1.21 ± 0.03 mmol/l respectively at the time of discharge from the hospital when glycaemic control was markedly improved and the changes in the values were statistically significant (p<0.01). Thus, they showed that when there was significant reduction of HbA1c % in NIDDM patients, the serum phosphate level rose significantly.

Raskin and Pak [17] studied 21 diabetic patients in whom treatment results ranged from “suboptimal” to “optimal” control and found that, as the mean plasma glucose decreased from 17.1 mmol/L to 5.2 mmol/L over 4 to 10days, serum phosphate level rose from 1.12 to 1.26 mmol/L (P < 0.001).

Gertner et al [18] studied mineral metabolism in 7 juvenile onset diabetic patients before and after achieving near normal glucose levels by 7 to 14 days treatment with a portable subcutaneous insulin infusion system. They found that as plasma glucose decreased from an average
of 221 mg/dL to 95.9 mg/dL, serum inorganic phosphate rose from 4.09 to 5.01 mg/dL (P < 0.001) due to a 25% rise in renal tubular threshold for phosphate. No change was noted in immunoreactive parathyroid hormone (PTH) and in 1, 25hydroxy-vitamin D.


The findings in the present study were supported by the reports of the above workers.

CONCLUSION

The present study was undertaken to determine the serum inorganic phosphate concentration and glycated hemoglobin percent in diagnosed type 2 diabetes mellitus patients and also to determine the correlation of serum inorganic phosphate concentration with glycated hemoglobin (HbA1c) % of the patients.

The study showed that the serum inorganic phosphate concentration was significantly decreased in the diagnosed type 2 diabetes mellitus patients in comparison to healthy individuals. Moreover, there was a significant reduction of serum inorganic phosphate concentration in cases whose glycaemic control was poor, which was reflected by increased HbA1c %.

There was a statistically significant negative correlation between serum inorganic phosphate concentration and HbA1c % in patients of type 2 diabetes mellitus in the present study. Therefore, these findings indicate that there was a definite reduction of serum inorganic phosphate concentration in type 2 diabetes mellitus patients as long as glycaemic control was not achieved and that reduction of serum inorganic phosphate concentration may have a contributing role in the progression of the disease and development of complications of diabetes.

The major issue arises whether to estimate serum inorganic phosphate levels routinely in all type 2 diabetes patients and whether to set a cutoff value of serum inorganic phosphate for good glycaemic control remains to be seen. Further studies with large sample size and longer duration of study with newer methods, which gives a more accurate picture of the actual serum inorganic phosphate and HbA1c status of the body, are required which might shed greater light in this regard.

REFERENCES


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