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RESEARCH ARTICLE

COMPARISON OF SALIVARY AND BLOOD GLUCOSE IN TYPE II DIABETICS AND HEALTHY ADULTS IN FASTING AND POSTPRANDIAL STATES

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ABSTRACT

Type II diabetes mellitus is considered a major cause of mortality and a large percentage of diabetics live without being diagnosed. (International Diabetes Federation, 2014) Diabetes is known to affect salivary composition and function. (Panchbai *et al*., 2010) This study was done to examine correlation between salivary and blood glucose in type II diabetics and healthy adults. Salivary and blood glucose levels were estimated and compared in both groups. Eighty adults in age group of 30 - 50 years were included in our study and divided into 2 groups - diabetics and healthy adults. Blood and saliva samples were obtained from subjects after an overnight fast and 2 hours postprandial. Blood samples were analysed with hexokinase enzyme using an automated analyser and saliva samples with glucose oxidase enzyme using a colorimeter. Difference in salivary glucose levels between groups was determined by unpaired t test and correlation between blood and salivary glucose levels by correlation test. Salivary glucose levels were higher in diabetics (9.77 ± 5.34 mg/dl) when compared to controls (5.77 ± 2.01 mg/dl) in the fasting state and the difference ($p<0.001$) was significant. Postprandial salivary glucose levels were significantly different between the two groups (13.65 ± 5.92 vs 10.57 ± 3.07mg/dl, $P<0.001$). Correlation between blood and salivary glucose levels was not seen in both groups. Salivary glucose levels are significantly higher in diabetics in fasting and postprandial states in our study. Hence, estimation of salivary glucose levels can be used as a mass screening method for diabetes in large populations.

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INTRODUCTION

Type II diabetes mellitus (DM) is characterized by insulin resistance (IR), impaired insulin secretion and increased glucose production. (Powers, 2008) It is estimated that there are 65.1 million diabetics in India according to IDF (International Diabetes Federation) in 2013. (International Diabetes Federation, 2014) In Asian countries, the diabetes burden is disproportionately high in young to middle-aged adults. (Chan *et al*., 2009) A greater risk of developing this disorder is seen among Asian Indians. (Satish *et al*., 2014) Urbanization is to be blamed for the rising prevalence of diabetes in India, which has even affected the rural areas. (Ramachandran *et al*., 2008) Visceral or central obesity is very common in type II DM, of which, IR is a prominent feature. Secondary pathophysiological changes in multiple organ systems is a consequence of DM. According to IDF, diabetes was responsible for 55 percent mortality under 60 years of age in Southeast Asia in 2013. (Ramachandran *et al*., 2008) By diagnosing diabetes early, long-term complications could be prevented. (Chan *et al*., 2009) Hence primary prevention of diabetes and its complications is of paramount importance. Saliva constitutes mainly of water, essential electrolytes, glycoproteins, antimicrobial enzymes and several other constituents like glucose and amylase. (Panchbai *et al*., 2010) Saliva is necessary for protection of the body from deleterious extrinsic influences. It is the first biological fluid to be affected by any change in eating habits or any environmental or physical changes. (Sariri *et al*., 2010)

There is a known association between diabetes mellitus and altered salivary composition and function. This disrupts the homeostasis of the oral cavity and makes it more prone for oral diseases. (Panchbai *et al*., 2010) Changes in the metabolism of lipids and proteins result in the development of vascular complications. (Belazi *et al*., 1998) Glucose, being a small molecule, moves through membranes of blood vessels. It then passes from the blood plasma via gingival sulcus to the gingival fluid, and reaches the saliva.

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(Vasconcelos *et al*., 2010) Earlier research has shown raised salivary glucose levels in diabetics. (Panchbai *et al*., 2010) Increased blood glucose may cause higher levels of salivary glucose. (Satish *et al*., 2014) Also, a decrease in mean glucose concentration was seen in the saliva of fasting subjects when compared to the control group. (Sariri *et al*., 2010)

IDF has reported that 46 percent of diabetes cases have gone undiagnosed worldwide in 2013. (International Diabetes Federation, 2014) Undiagnosed diabetes can cause progressive microvascular and macrovascular damage. If diabetes is left undiagnosed and untreated, it will result in a lower life expectancy. Therefore, innovative and improved methods of testing for the disease is crucial in lowering the disease burden. Self monitoring of blood glucose has been tremendously useful for monitoring diabetes. However, many patients find the testing vexatious as it is painful, inconvenient, messy, embarrassing and most importantly, expensive. (Harris *et al*., 1987) The number of studies that evaluate glucose concentration in saliva is limited. Contradictory results have been obtained from these studies. Very few studies have been done to study the relationship between salivary and blood glucose in fasting and postprandial states in India. If a correlation exists, estimating salivary glucose levels could be used as a non-invasive method for determining glucose levels in diabetics.

MATERIALS AND METHODS

This comparative study was done to determine and compare salivary glucose levels in diabetics and healthy adults using the method of colorimetry. The relationship between blood glucose and salivary glucose levels was also assessed. Subjects with type II diabetes and healthy controls (n=80) (40 in each group) were recruited for the study from outpatients attending KIMS Hospital outpatient department, Bangalore. The subjects were divided into two groups: GROUP I – Experimental group which comprised of 40 patients of both genders with type II diabetes mellitus in the age group of 30 – 50 years and GROUP II – Control group which comprised of 40 healthy non–diabetic individuals who were age and sex matched. Patients who had been diagnosed with type II diabetes mellitus were included in the experimental group and non- diabetic healthy individuals in the control group. Written informed consent was taken from all subjects. The study was approved by the Ethics Review Committee of the Institution.

The study was conducted in the lab in the Department of Biochemistry, Kempegowda Institute of Medical Sciences, Bangalore. All subjects underwent anthropometric assessment which included recording of height using a stadiometer and also weight to the nearest 100gms. BMI was calculated using the formula weight divided by square of height (kilograms per square meter). Estimation of glucose levels of saliva and blood was done for all the subjects. Those with severe diabetic complications, other systemic illnesses, those on medications other than those for Diabetes mellitus, smokers, tobacco users, edentulous individuals, those with history of prior surgery of salivary glands, being treated with radiotherapy of head and neck region, those with Sjogren's syndrome, rheumatoid arthritis or SLE and secondary diabetes were excluded from the study.

For salivary glucose measurement, samples of saliva were collected on empty stomach (after 12 hours of fasting) and 2 hours postprandial. It was collected by spitting method between 8am and 11 am (Subjects should not have had any meal or practised any oral hygiene 90 minutes before procedure). After gargling their mouths with about 5 ml of distilled water for about 2 minutes, un-stimulated whole saliva samples (5 ml) were collected in clean, dry sterile tubes. Samples were frozen at -20ºC and then thawed. After that, samples were centrifuged at 3000rpm for 20 minutes. The supernatant was stored at – 18°C until it was used for determining glucose content. They were analyzed within 48-72 hours of collection. Each assay was repeated three times and the data obtained were expressed as mean ± SD (Standard deviation) of the three values.

Salivary glucose estimation was done by enzymatic colorimetric test method using a manual colorimeter (CL157). Glucose levels of saliva were measured using the Glucose Oxidase kit (Agappe Diagnostics Limited, Kerala, India) in colorimeter. The sensitivity of the assay was 77.8% and specificity was 100.00%. The level of glucose in saliva was measured using an enzymatic method based on the oxidation of glucose by glucose oxidase (GOD) followed by determination of resulting H_2O_2 in the presence of peroxidase (POD). 1000µl of this reagent was taken in three test tubes marked 'Blank', 'Standard' and 'Test.' 10µl of glucose standard (100mg/dl) and 10µl of sample were taken in the test tubes marked 'Standard' and 'Test' respectively. These three test tubes were then incubated at 37^{o}C for 20 minutes. 'Blank' was used to zero the spectrophotometer at 505nm. Absorbance values of 'Standard' and 'Test' were measured at 505 nm.

For blood glucose measurement, blood samples were collected on empty stomach (after 12 hours of fasting) and 2 hours postprandial. Blood glucose estimation was done by enzymatic colorimetric test method using an automated analyser (cobas e501). Glucose levels in these samples were measured using the Hexokinase Kit (Agappe Diagnostics Limited, Kerala, India) in an automated analyser. 1000 µl of this reagent was taken in three test tubes marked 'Blank', 'Standard' and 'Test.' 10 µl of glucose standard (100mg/dl) and 10 µl of sample were taken in the test tubes marked 'Standard' and 'Test' respectively. These three test tubes were then incubated at 37^{o}C for 20 minutes. 'Blank' was used to zero the spectrophotometer. Absorbance values of 'Standard' and 'Test' were measured at 505 nm. The results are presented as mean ± SD. The significance of any difference was tested with t-test and Mann-Whitney test wherever appropriate. Differences were considered statistically significant for P values < 0.05. Correlation between blood and salivary glucose values were determined by Spearman's rank correlation test.

RESULTS

The mean age of the diabetic group was 42.03 ± 5.77 years and the mean age of the control group was 39.95 ± 6.43 years. While the mean BMI of the experimental group was 25.37 ± 2.57 kg/m^2, the mean BMI of the control group was 24.46 ± 3.33 kg/m^2 (Table 1).

Table 1. Characteristics of subjects

	Diabetic group	Control group
Males (n)	17	12
Females (n)	23	28
Age (years)	42.03 ± 5.77	39.95 ± 6.43
BMI (kg/m^2)	25.37 ± 2.57	24.46 ± 3.33

Values are expressed in mean ± SD

Table 2. Blood glucose and salivary glucose levels of the groups

	Diabetic group	Control group	*P* value
Fasting blood glucose (FBG)(mg/dl)	147.93 ± 65.50	88.00 ± 12.48	<0.001*
Postprandial blood glucose (PPBG)(mg/dl)	224.40 ± 90.25	126.73 ± 30.85	<0.001*
Fasting salivary glucose (FSG)(mg/dl)	9.77 ± 5.34	5.77 ± 2.01	<0.001*
Postprandial salivary glucose (PPSG)(mg/dl)	13.65 ± 5.92	10.57 ± 3.07	0.001*

*Statistically significant difference

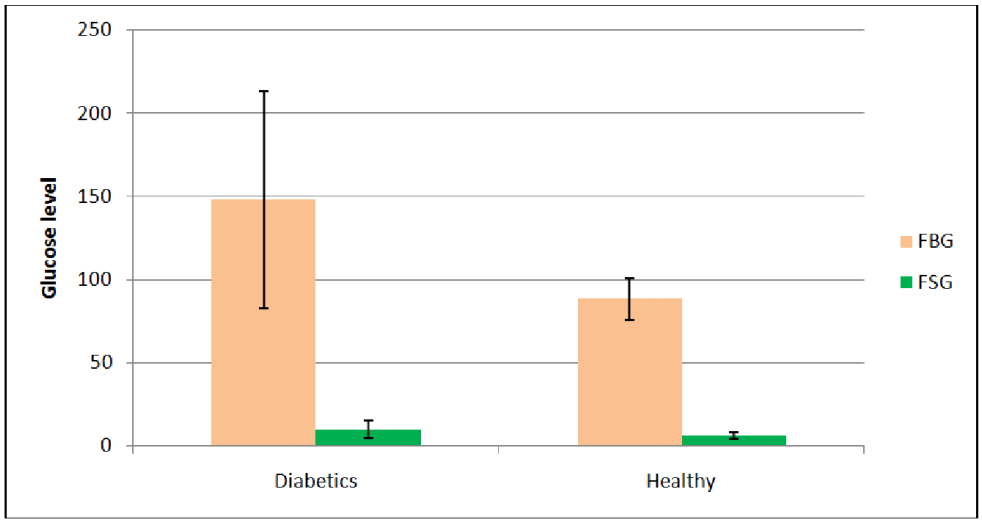

Figure 1. Mean Fasting blood glucose (FBG) and Fasting salivary glucose (FSG) in the Groups

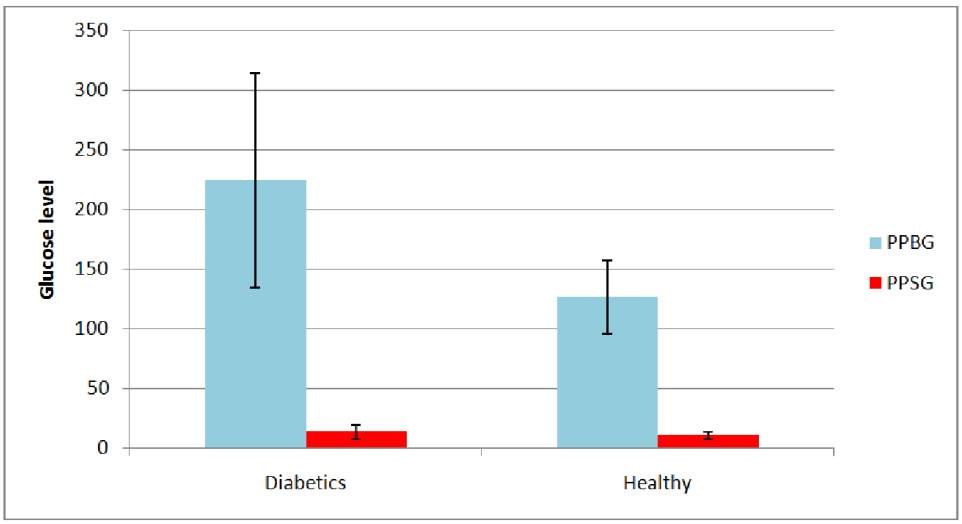

Figure 2. Mean Postprandial blood glucose (PPBG) and Postprandial salivary glucose (PPSG) in the groups

As shown in Table 2 (Figures 1 & 2), the mean salivary glucose concentration in the fasting state was 9.77 ± 5.34 mg/dl for the study group and 5.77 ± 2.01 mg/dl in the control group and this difference was statistically significant (*P* <0.001). The mean salivary glucose level in the postprandial state for the diabetic group was 13.65 ± 5.92 mg/dl and in healthy adults, 10.57 ± 3.07 mg/dl, a statistically significant difference *P* <0.001). (When considering blood glucose, the experimental group exhibited a mean of 147.93 ± 65.50 mg/dl, while the mean of the control group was 88.00 ± 12.48 mg/dl in the fasting state; also a statistically significant difference

(P <0.001). In the postprandial state, the mean blood glucose level in diabetics was 224.40 ± 90.25 mg/dl and in healthy adults, 126.73 ± 30.85 mg/dl, a statistically significant difference (P =0.001).

No statistically significant association was found between the salivary and blood glucose in both experimental and control groups in both fasting and postprandial states.

DISCUSSION

Diabetes mellitus is a constellation of abnormalities caused by insulin resistance and deficiency. (Ganong, 2012) The onset of diabetes in Asia is at lower BMI levels and younger ages when compared to the Western population although, the average BMI in Asian populations is still relatively low. Some of the factors that contribute to the rise in the diabetes epidemic in Asians are "normal-weight metabolically obese" phenotype, high prevalence of smoking, excessive alcohol intake, high intake of refined carbohydrates and dramatically decreased physical activity levels. Poor nutrition during intrauterine and in early life followed by over nutrition later in life may also play a role in Asia's diabetes epidemic. (Hu, 2011)

It is possible that Asians are more genetically susceptible to insulin resistance and diabetes than Whites. (Dickinson *et al*., 2002) In the present study, the mean BMI of the study group was 25.37 ± 2.57 kg/m^2 and that of the control group was 24.46 ± 3.33 kg/m^2 and there was no significant difference between them (P=0.204) (Table 1). The subjects in this study were age-, sex- and BMI-matched. A majority of the people with diabetes are in the 45-64 year age range in developing countries whereas the majority in developed countries are above 64 years of age. (Wild *et al*., 2004) A greater number of people affected by DM were in the age group of 46-50 years in this study. A temporal shift in the age of diagnosis of diabetes supports the finding of CURES (Chennai Urban Rural Epidemiology Study). (Mohan *et al*., 2007) Studies that have shown an increase in prevalence of diabetes have also reported a very high prevalence of undiagnosed diabetes in the community. (Ramachandran *et al*., 2001) Hyperglycaemia is a paramount consequence of DM and in due time, there is widespread multisystem damage. Early identification of at-risk individuals using simple screening methods like the Indian Diabetes Risk Score (IDRS) and appropriate changes in lifestyle would greatly help in preventing or postponing the onset of diabetes and thus reducing the burden on the community and the nation as a whole. (Mohan *et al*., 2005; Mohan and Anbalagan, 2013)

Since no cure is known for diabetes, patients depend on constant monitoring to maintain normal blood glucose levels. Investigations that are conventionally done include assessments of plasma concentrations of glucose, glycated hemoglobin and fructosamine and urine testing. The oral hypoglycemic agents commonly used to manage DM are sulphonylureas (stimulate the secretion of insulin and increase the number of insulin receptors if there is some endogenous insulin production) and biguanides (decreases hepatic gluconeogenesis and increases peripheral utilization of glucose). (Manfredi *et al*., 2004) All diabetics in the present study were on oral hypoglycemic agents.

A number of non invasive devices have currently been researched to provide diabetics an alternative. Saliva is currently being researched for use in the diagnosis of Sjogren's syndrome, dental caries, carcinomas, endocrine disorders, pregnancy, steroid and protein analysis, HCV (Hepatitis C virus), HIV (Human Immunodeficiency Virus), Hepatitis B antibodies, Cystatin A in GCF in periodontitis. Furthermore, analysis of saliva provides vital information about the functioning of various organs in the body.[21] Saliva offers superior advantages over the other innovative NI techniques that have been developed because it can be collected non - invasively by individuals with modest training. Moreover, saliva might provide a cost effective approach for screening large populations.

In the present study, FBG levels in diabetics ranged from 60–227 mg/dl with a mean value of 147.93 ± 65.50 mg/dl. These values were significantly higher than the non – diabetic group where highest value was 114 mg/dl (P<0.001). The mean FSG (Fasting salivary glucose) was found to be higher in diabetics (9.77 ± 5.34 mg/dl) than the non – diabetic group (5.77 ± 2.01 mg/dl) and this difference was found to be statistically significant (P<0.001) (Table 2) (Figure 1).

Many studies have been performed to investigate the effect of fasting on the metabolism of glucose. Thorstensson *et al*. (1989) observed FPG level in diabetics to be greater than non-diabetics. Sashikumar *et al*. (2010) reported that FSG was greater in the study group when compared to the control group. Mahdavi *et al*. (2012) found that the mean FPG level in their study group was significantly higher in diabetics than in controls. They also observed that the average FSG level in the diabetic group was significantly higher than in non-diabetic subjects (P=0.0001). The mean PPBG (Postprandial blood glucose) was 224.40 ± 90.25 mg/dl in diabetics and 126.73 ± 30.85 mg/dl in healthy adults in our study. This increase in blood glucose levels in diabetics when compared to non – diabetic group was statistically significant (P<0.001). The mean PPSG (Postprandial salivary glucose) (13.65 ± 5.92 mg/dl) was found to be significantly higher in the study group than in the control group (10.57 ± 3.07 mg/dl) and this difference was found to be statistically significant (P=0.001) (Table 2) (Figure 2).

Vasconcelos *et al*. (2010) found in a comparative study of the concentration of salivary glucose in type II diabetic patients in relation to blood glucose that diabetics exhibited a statistically significant higher blood glucose levels than the non – diabetic group (P= 0.000). They also found that the concentration of salivary glucose in diabetic patients was significantly higher than in non diabetic individuals (P=0.036). Study by Amer *et al*. (2001) on 25 age and sex matched diabetic and non diabetic subjects has shown that glucose was found only in salivary sample of patients with diabetes mellitus, while the salivary sample of non-diabetic subjects did not show the presence of glucose.

Glucose is an essential source of energy which can be obtained through food intake or produced within the cells of the body. The normal levels do not significantly affect oral health or support the growth of micro organisms. However higher

salivary glucose levels favour the proliferation of micro organisms and enhance their colonization on teeth and oral mucous membranes.

An association between diabetes mellitus and alterations in the oral mucosa has been observed in experimental studies and clinical practice (Babu *et al*., 2014; Ivanovski *et al*., 2012; Navalkar and Bhoweer, 2011). A study done by Murrah, Crusson and Sauk (Murrah *et al*., 1985) showed that changes were seen associated with the basement membrane of the parotid gland of diabetic patients. The elevated glucose levels observed by them in saliva also confirms the effect of diabetic membranopathy, which leads to an increased percolation of glucose from blood to saliva, thus affecting the salivary composition in these patients. This could explain the increased levels of salivary glucose in diabetics. The mean PPSG was found to be higher than the mean FSG in Diabetics and this difference was found to be statistically significant (P<0.001). The mean PPSG was found to be higher than the mean FSG in healthy samples and this difference was found to be statistically significant (P<0.001) (Table 2). Some mechanisms contribute to the rise in salivary glucose levels after meals. The higher level of salivary glucose in diabetics in postprandial state suggests that metabolism in the study subjects increases the basal levels of salivary glucose. This suggests the effects of metabolism on simple and complex carbohydrates which form the major portion of the diet of South Indians.

There was no correlation seen in the relation between blood glucose and salivary glucose in both fasting (r=0.054, *P*=0.740 & r=0.167, *P*=0.304) and postprandial (r=0.007, *P*=0.968 & r=0.051, *P*=0.753) states in both groups. Sashikumar *et al*. (2010) also reported there was no significant relation between FSG and FPG. Panchbai *et al*. (2010) found that their results were insignificant for any correlation between post meal BGL (Blood glucose level) and random BGL for the control group as well as for the healthy non-diabetic group. Darwazeh *et al*. (1991) observed no significant correlation between blood and salivary glucose levels in controls (r=0.21, *P*>0.05) No correlation probably implies that separate mechanisms are involved in the metabolism of salivary and blood glucose. The correlation between SGL (salivary glucose levels) and BGL cannot be validated until the salivary glucose estimation is standardised. It is yet to be known how the intraglandular carbohydrate mechanism influences the SGL. Moreover, glucose being a nutrient source for the microbes in the oral cavity, a portion of the glucose in saliva might be utilized in this manner and this could thus influence the total glucose content in saliva. The role of insulin in regulating salivary glucose also needs to be studied.

A positive correlation was seen between blood glucose levels in both fasting (r=0.371, *P*=0.018) and postprandial (r=0.333, *P*=0.036) states and BMI in diabetic group. McAdams *et al*.[32] observed that self-reported and measured BMI values were equally correlated with fasting blood glucose (r=0.43). This could mean that maintenance of body weight does play a key role in regulation of blood glucose. No correlation was seen in the relationship between blood and salivary glucose levels in both fasting and postprandial states and BMI in healthy non-diabetic group in the present study (*P*>0.05). The results we acquired are supported by some studies but differ from those obtained by other researchers. This may be due to the diversity in the selection criteria of the samples and the type of design of each study, differences in the methods employed in collection of saliva and variation in age of the subjects studied, and varying levels of metabolic control in diabetic patients.

Thus it can be inferred from the present study that levels of salivary glucose were significantly higher in both fasting and postprandial state. No correlation was seen between blood and salivary glucose levels in our study. Saliva can be used as a screening method, as per these findings, as salivary glucose levels were found to be significantly higher in diabetics. Early identification of people at risk of developing type II diabetes will help in preventing progression from prediabetes to frank diabetes and its complications. If biomarkers are identified by non–invasive methods, a greater number of people will participate in the screening of diseases.

Further studies will help in development of devices which employ non-invasive methods that are sensitive enough to estimate glucose levels in saliva and thus provide accurate results. Portable glucose monitoring systems have been developed by Zhang *et al*. (2014), Mucci *et al*. (2014) and other researchers, which are nanocomposites and determine salivary glucose levels by spectrophotometry. These devices are enabled to transmit data and spectral imagery across the globe which is an added advantage.

Limitation

The sample size in this study was small. If larger populations are studied in the future, more information can be obtained about the relationship between blood glucose and salivary glucose levels.

Conclusion

Salivary glucose levels are significantly higher in diabetics in fasting and postprandial states in our study. Hence, estimation of salivary glucose levels can be used as a mass screening method for diabetes in large populations. Correlation between blood and salivary glucose levels was not seen in the present study. Further studies with greater sample size might contribute to using salivary diagnostics in diabetics if a correlation is found. This would be beneficial for diabetics especially juvenile diabetics and patients with gestational diabetes mellitus as multiple pricks will not be required for drawing blood and multiple sampling is possible. Also, non-invasive techniques of glucose estimation might help to reduce the number of undiagnosed diabetes cases.

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