



A Study between Diabetes type-1 and Dental cavity in macro-mineral Elements in Adults and with Phenylketonuria in Childrens.

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Abstract

This study will carry on to evaluate the possible protective role of macro-minerals elements like adequate level of Calcium, Phosphate ,fluoride & pH and alkaline phosphatase levels in dental cavity of diabetic adults. The aim of this study was to investigate the oral parameters that influence the caries risk and risk of developing periodontal disease in children with PKU and type 1 diabetes. The parameters to be assessed were the dental and oral hygiene status and oral microorganisms in children with diabetes, PKU and in healthy childrens.

Keywords: Dental cavity; Serum calcium, phosphate, fluoride and lactic acid, phenylketonuria.

Introduction

The present study aims to provide an insight into the oral status of children& adults suffering from two different metabolic diseases. At the same time it seeks to answer questions on whether the dietary regulations of the children have an effect on their dental health and whether there is an alteration in their oral micro flora, putting the children at a higher or lower risk for developing dental caries and periodontal disease (Mumghamba EG.,2006).

Diabetes is a metabolic disorder i.e. principally classified into type 1 and type 2 diabetes. Traditionally, children suffering from type 1 diabetes had to follow a diet restricted in carbohydrates in order to maintain normal blood sugar levels (Lalla E et al., 2010).The intake of carbohydrates was aligned with the insulin regime. Advancements insulin therapy regimes have led to the relaxation of dietary restrictions & type 1 diabetics are now able to follow a diet quite similar to normal healthy individuals (Kakade SP .,2014).

Dental cavity, is an infection, bacterial in origin, that causes demineralization and destruction of the hard tissues (enamel, dentin and cementum), usually by production of acid by bacterial fermentation of the food debris accumulated on the tooth surface. If demineralization exceeds saliva and other remineralization factors such as from calcium and fluoridated toothpastes, these hard tissues progressively break down, producing dental cavities (Shahrabi M, et al.,2008).

The dental status was assessed by the dmfs / DMFS Index and the gingival health and oral hygiene was evaluated using the Papillary Bleeding Index (PBI) . i.e. the aim of this study was to investigate the oral parameters that influence the caries risk and risk of developing periodontal disease in children with phenylketonuria (PKU) and type 1 diabetes (Chávarry NG et al.,2009). The parameters to be assessed were the dental and oral hygiene status,

gingival health and oral microorganisms in children with diabetes, PKU and healthy children.

It is often essential for children suffering from metabolic diseases to follow a strict diet to keep the disorder under check and to be able to develop and function normally. Phenylketonuria (PKU) is a metabolic disorder in which the patients present with an absence or deficiency of the enzyme phenylalanine hydroxylase which is essential to metabolise the amino acid phenylalanine into the amino acid tyrosine (Twetman S et al.,2002) (Fig. no.01) Uncontrolled, the

disease can lead to the accumulation of phenylalanine in the blood and brain causing disabilities. In order to keep the ingestion of phenylalanine to a minimum, children with PKU follow a special low protein diet. At the same time, their diet is rich in carbohydrates and the phenylalanine -free formula drinks have a high pH (Vijayaprasad KE et al.,2010). The frequency of ingestion of these carbohydrates is high and therefore, the risk for the development of caries in children suffering from PKU is considered to be high (De Marco et al.,2015).

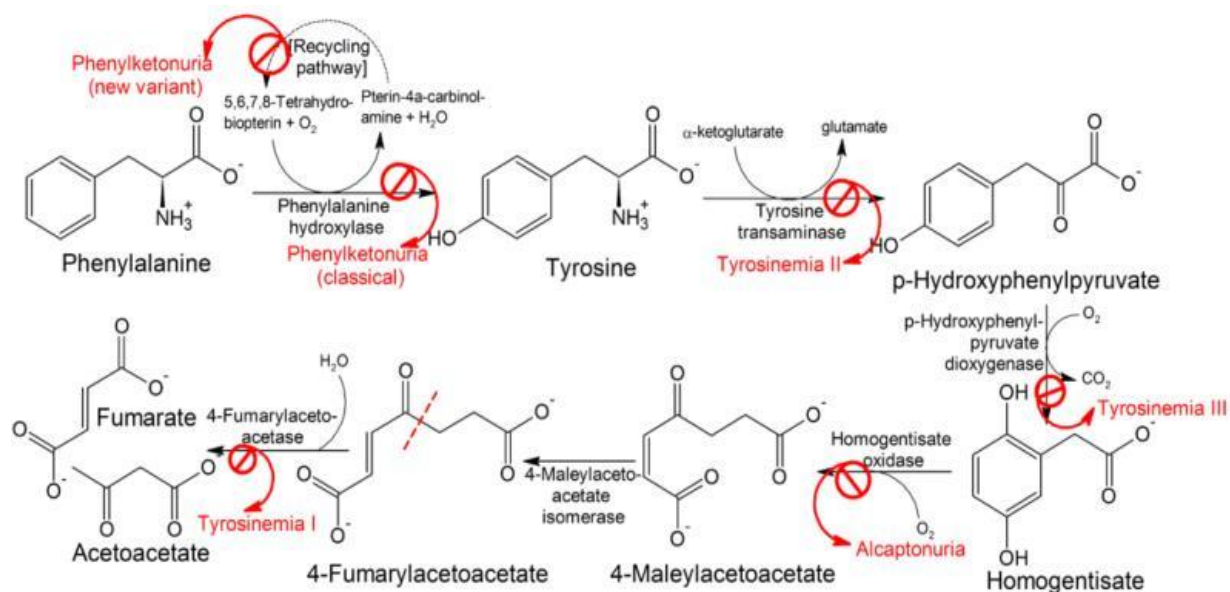


Figure 01 Defect in phenylketonuria

Materials and Methods

Total of 100 subjects of either sex, aged between 20-60 were selected. Decayed, missed and filled teeth (DMFT) were used as indices for scoring the dental cavity and were distributed into 4 groups on the basis of DMFT indices as 30-40 (Group I), 41-50 (Group II), 51-60 (Group III) and more than 60 (Group IV), while the control subjects had DMFT index equal to or less than 3. Serum was collected and pH, calcium, phosphate, fluoride and lactic acid were analyzed. Total children's are selected between the ages of 3 and 18 years were recruited for each group in the study. The total sample consisted of 238 children. The PKU group had 38 children and both the diabetic and healthy control group comprised of 100 children each. Demographic data was collected with the help of a standard questionnaire.

All the subjects were free from any systemic illness and were not taking any caries preventive regimen like fluoride toothpaste, fluoride rinses or NaF/calcium tablets. Subjects who gave improper history about

missed tooth or suffering from any type of Xerostomia or having any oral inflammatory problems were not included in the study. Dental examination is done with the assistance of dentist under natural light source. Decayed, missed and filled teeth (DMFT) were used as index for scoring the dental caries (Busato IM et al.,2012). All subjects were distributed into 5 groups (Table-1) each having twenty individuals. Like group 1 with DMFT index 30-40, group 2 with DMFT index 41-50, group 3 with DMFT index 51-60 and group 4 with DMFT index more than 60, while the control subjects have the DMFT index equal or less than 3.

A cross-sectional study for children's was conducted and involved the examination of patients from 3 groups Children suffering from PKU (Group A) and type 1 diabetes (Group B) . The control group (Group C) consisted of healthy children. Children between the ages 3 and 18 years were recruited for the study. The total sample consisted of 238 children. Both Group B and C comprised of 100 children and Group A had 38 children. The patients were matched for gender and age.

10 mL of venous blood sample was drawn after applying a tourniquet, followed by proper aseptic precautions with a sterile disposable plastic syringe without any anticoagulant. A drop of blood was put on the electrode of pH meter from the novel of syringe carefully for blood pH determination. 0.5 mL of blood was immediately put into sterile bottle containing 0.5 mg of EDTA (Ethylene Diamine Tetra Acetic acid) powder, shaken gently and stoppered. This blood is used within 24 hours for the estimation of lactic acid.

The blood in the syringe was covered, labeled and transferred in an ice box to the laboratory. Blood sample was centrifuged for 15 minutes at 3000 rpm. The hemolyzed samples were discarded. The supernatant layer of serum was then separated and poured in labeled glass bottles and stored in deep freezer at -20°C. The serum pH was measured electrometrically with the glass electrode by digital pH meter HI 8014 (Hanna Instrument, USA). After calibration and temperature adjustment the bulb of glass electrode was immersed in a drop of serum sample and pH was noted from the screen of digital pH meter. The serum calcium was estimated calorimetrically by using kit (Ref # 995936) supplied by Quimica Clinical Aplicada SA Aposta Spain.

Serum inorganic phosphorus was measured by colorimetric method using kit, cat # KC 120 supplied by Clonital Italy. Serum fluoride was also measured by colorimetric method using alazerine and zirconium dye. The fluoride was analyzed by the Magregian, Haier method cited by Farber in which the fluoride reacts with dye lake, dissociating a portion of it into a colorless complex anion (ZrF₆) and the dye. As the amount of fluoride increased, the color produced becomes progressively lighter or different in hue depending on the reagent used (Rosin-Garget K et al., (2001). The student's "t-test" was used to compare the serum calcium, phosphate and fluoride among the control and diseased groups.

Results and Discussion

One hundred individuals were divided into five groups according to their DMFT index (Table-1). The distribution of sex is approximately equal in all groups. The base line comparison of mean values of age, DMFT, index and number of brushing per day (Table-2) shows a significant decrease in number of brushing and significant increase in DMFT index in all groups when compared to control.

Table 1: Distribution of control and patients in groups. (According to the DMFT index)

Group	DMFT index	Distribution of subjects	Sex	
			Male	Female
Control	<30	20	13	7
Group – I	30-40	20	11	9
Group – II	41-50	20	11	9
Group – III	51-60	20	10	10
Group – IV	> 60	20	10	10

Table 2: Baseline comparison of personal data of the control and patients.

Groups	Age (years)	DMFT Index	Brushing (No. of times/day)
Control	23.9	1.35	2.05
(n=20)	+1.623	+0.208	+0.05
Group – I	27.75	6.3*	1.6*
(n=20)	+1.680	+0.291	+0.11
Group – II	28.25	12.15*	1.05*
(n=20)	+1.769	+0.099	+0.135
Group – III	31.7*	19.8*	0.5*
(n=20)	+1.818	+0.47	+0.114
Group – IV	31.95*	26.95*	0.15*
(n=20)	+1.59	+0.364	+0.08

Values are expressed as mean + SEM, * P < 0.001 as compared to control.

Table 3 shows the comparison of the mean values of serum pH, calcium, phosphate, fluoride and lactic acid between control and all groups. In group I there is a significantly decreased level of serum, calcium and fluoride and significantly increased level of lactic acid when compared to control subjects (P<0.001). in

group II, III and IV serum, calcium, phosphate and fluoride observed decreased significantly and a significant increased in serum lactic acid when compared to control subjects (P<0.001). No significant change is observed in serum pH of all groups when compared to control group.

Table 3: Comparison of serum pH, calcium, phosphate, fluoride and lactic acid between control and groups.

Parameters	Control (n=20)	Group I (n=20)	Group II (n=20)	Group III (n=20)	Group IV (n=20)
pH	7.412	7.407	7.417	7.419	7.418
	+0.005	+0.006	+0.005	+0.004	+0.005
Calcium (mg/dl)	10.275	9.72**	9.1**	8.6**	7.955**
	+0.154	+0.128	+0.127	+0.139	+0.115
Phosphate (mg/dl)	4.22	4.03	3.59**	3.005**	2.295**
	+0.117	+0.099	+0.047	+0.032	+0.059
Fluoride (mg/dl)	4.4	2.295**	1.615**	0.76**	0.58
	+0.393	+0.317	+0.713	+0.044	+0.069
Lactic acid (mg/dl)	7.45	11.765**	15.32**	18.14**	22.875**
	+0.413	+0.809	+0.695	+0.794	+0.956

Values are expressed as mean + SEM., ** P<0.001 as compared to control.

Table 4 shows the intergroup comparison of mean values of serum pH, calcium, phosphate, fluoride and lactic acid. A significantly decreased serum calcium and phosphate and increased lactic acid were observed in group II, III and IV when compared to group I whereas fluoride was significantly decreased in group II and IV when compared to group I. When group III and IV were compared with group II, the decreased serum calcium, phosphate and increased lactic acid were observed. In contrary when group IV compared with group III, significantly decreased level of

calcium, phosphate, fluoride and increased lactic acid were observed. In group II serum calcium and phosphate were significantly decreased while lactic acid was significantly increased when compared to group I (P<0.001). In group III and IV serum calcium, phosphate and fluoride were decreased significantly while lactic acid was increased significantly when compared to group I (P<0.001). In group III serum calcium and phosphate were significantly decreased and lactic acid is significantly raised when compared to group II (P<0.05).

Table 4: Inter group comparison of serum pH, calcium, phosphate, fluoride and lactic acid.

Parameters	Group I (n=20)	Group II (n=20)	Group III (n=20)	Group IV (n=20)
pH	7.7407	7.417	7.419	7.418
	+0.006	+0.005	+0.004	+0.005
Calcium (mg/dl)	9.72	9.1**	8.6**†	7.955**††ÅÅ
	+0.128	+0.127	+0.139	+0.115
Phosphate (mg/dl)	4.03	3.59**	3.005**††	2.295**††ÅÅ
	+0.09	+0.047	+0.032	+0.059
Fluoride (mg/dl)	2.295	1.615	0.76**	0.58**Å
	+0.317	+0.713	+0.044	+0.069
Lactic acid (mg/dl)	11.765	15.32**	18.14**†	22.875**††ÅÅ
	+0.809	+0.69	+0.794	+0.956

Values are expressed as mean + SEM.* P < 0.05, ** P < 0.001 as compared group I vs. all groups.
 † P < 0.005, †† P < 0.001 as compared group II vs. III and IV. Å P < 0.02, ÅÅ P < 0.001 as compared group III vs. IV.

Both dental cavity (periodontitis) and diabetes mellitus are frequent chronic diseases and generate enormous costs for the public health care system. Numerous studies, review articles and meta-analyses indicated a mutual influence between periodontitis and diabetes mellitus. The mechanisms, whereby diabetes may negatively influence periodontal health, are primarily based on the impaired local immune defense and a reduced renewal of the periodontal tissues. Moreover, higher levels of advanced glycation end products (AGE) can be found in the dental cavity of diabetics compared to non-diabetic subjects. The interaction between AGEs and collagen generates highly stable collagen macromolecules, that are resistant to physiologic enzymatic degradation. Hence, the renewal of all periodontal tissues is effectively compromised in diabetic subjects, especially when glycemic control is poor. These phenomena explain in part why diabetic patients are three times more likely to develop periodontitis than non-diabetic subjects.

The role of serum calcium, phosphate and fluoride & pH in dental caries has been the point of interest since the mid of this century by many oral hygienist in the field of oral biochemistry. The early work of Stephan regarding the estimation of salivary pH had showed that the pH of saliva remained below the critical level of 5.5 in dental caries of diabetic patients, than the caries free people. Another study carried out by Abelson and Mandel demonstrated that the saliva exert its major influence on caries initiation by means of plaque formation rather than by direct contact on

the tooth surface, they showed that plaque pH fall was greater in caries susceptible subjects. However this study did not show any significant change in the blood pH with the progression of disease.

Conclusion

The study is carry on by previous workers revealed that the calcium ions are present normally in dental plaque bound to matrix and other proteins attracting phosphate and fluoride as counter ion, other phosphate and fluoride occurs intracellularly. All three ions occur as an inorganic mineral in serum and are in continuous exchange phase with the saliva over the dental plaque. This is responsible for the “pool” or “reservoir” of calcium, phosphate and fluoride in dental plaque and also maintains their saturation. These observations are quite identical with our study as levels of serum calcium, phosphate and fluoride are significantly low in dental caries patient in comparison to the control..

A comparison of the dmfs index for the primary dentition revealed that the children in group A had an astonishingly high value as compared to the other two groups. A statistically significant difference was found between the three groups. Primary teeth of the children in group A showed a mean (\pm Standard deviation) dmfs index value of 4.18 (\pm 7.46) whereas those of group B and C showed values of 1.38 (\pm 5.33) and 3.86 (\pm 7.68), respectively. Thus the children in group B demonstrated the lowest mean dmfs index values.(table 5)

Table no.5 Dmfs Index :

Group	N	Minimum	Maximum	Mean	Standard Deviation
A	37	0	32	4.18*	7.46
B	100	0	50	1.38*	5.33
C	100	0	56	3.86*	7.68

Table 5: dmfs index values for groups A, B and C. * indicates statistically significant difference between the groups, p 0.05

The results of the present research showed that the mean dmfs index value in the group of children suffering from PKU was statistically significantly higher than in the healthy children and the diabetic children.

Our study quite clearly gives the information that there is significant fall in serum calcium, phosphate and fluoride as the disease process advances.

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