Research article Available online www.ijsrr.org ISSN: 2279–0543

International Journal of Scientific Research and Reviews

Association of Sweet Perception with Glut2 Polymorphism in Type II Diabetes Mellitus Patients with Caries.

Sridevi N.[*1], Jaya Prakash Thumu[1], Anil Kumar[2] and A.V.Rajanikanth[3]

[1,*]Department of Conservative Dentistry and Endodontics, Rama Dental College, Hospital and Research Centre, Kanpur (UP) India.
[2]Central Research Laboratory, Rama Medical College Hospital and Research Centre, Mandhana, Kanpur, (UP) India.
[3]Department of Prosthodontics, Rama Dental College, Hospital and Research Centre, Kanpur (UP) India.

ABSTRACT

GLUT2 gene polymorphism plays a significant role in Type 2 diabetes patients. It has been investigated that single nucleotide polymorphisms (SNPs) in the GLUT2 gene affecting taste preference were recently associated with caries risk in Type 2 diabetes patients. The aim of the present study was to evaluate the association of GLUT2 gene polymorphism with sweet perception in dental caries susceptibility in Type II Diabetes mellitus patients in north Indian population.

120 subjects aged 30-70 years participating in this study were divided into two groups, GROUP I (n=60) included Type II diabetes mellitus patients with caries and GROUP II (n=60) included non-diabetic patients with dental caries. Sweet perception in all the individuals was measured by using a 9-point hedonic scale and the sweet liking score was created by taking the mean of the liking given by each individual to specific sweet food and beverages. Caries prevalence was assessed by using DMFT (decayed, missing, and filled teeth), DMFT + X-ray. GLUT2 (Thr110Ile, rs5400) genotypes were determined using ECOR1 assay.

In Type 2 diabetic patients a higher sweet liking scores (7.195 ± 0.4656), DMFT+X ray (7.25 ± 1.035**)** and DMFT (6.07 ± 0**.**880) scores were observed when compared with non-diabetic patients (p=0.001). Higher sweet liking score (7.07 ± 0.57), DMFT+X ray (6.98 ± 1.43), DMFT (5.72 ± 1.09) (p=0.001) were observed in AA Genotype than their counterparts and no significant differences were found in this regard between AG and GG genotype. In conclusion polymorphism of GLUT2 gene contributed to caries prevalence and highlighted the role of sweet liking as a predictor of caries risk.

KEYWORDS: GLUT2 gene, Type 2 diabetes, Sweet liking score, Dental caries, Gene polymorphism.

*Corresponding author

Sridevi. N

Ph.D. Scholar, Department of Conservative Dentistry and Endodontics,

Rama Dental College, Hospital and Research Centre, Kanpur, Rama University India.

E Mail i.d– sridevinandamuri98@gmail.com. ph.no-8887856069

INTRODUCTION

Dental caries is one amongst the most common perpetual ailments to which most of the world's population is vulnerable all through their lifetime. Various organic acids such as lactic, acetic, and formic acids produced by the acidogenic bacteria dissolve the calcified tissues of the tooth thus resulting in dental caries[1]. Several physical, biological, environmental factors such as increased quantities of cariogenic bacteria, inadequate fluoride consumption, decreased salivary secretion and poor oral hygiene factors lead to high risk for caries[2]. There are many possibilities to intervene in this continuing process to arrest or reverse the progress of the lesion. Remineralization is the natural repair process for non-cavitated lesions, and relies on calcium and phosphate ions assisted by fluoride to rebuild a new surface on existing crystal remnants in subsurface lesions remaining after demineralization[3].

Diabetic patients are more susceptible to dental caries and periodontal diseases. Resistance exhibited by the body cells to effects of insulin leads to the most prevalent type of diabetes, Type 2 diabetes mellitus[4]. This disease is associated with various risk factors such as increasing age, genetics, and obesity, lack of physical activity, stress and increased consumption of sweetened beverages[5, 6]. Previous studies had revealed patients with Type 2 diabetes mellitus were prone to a high risk of dental caries which can be attributed primarily to increased concentration of glucose in the blood[7].

Genetic play a key role in determining caries risk in the population, but the effect of genes in caries susceptibility is not much explored.GLUT2 gene is primarily involved in glucose homeostasis[8]. This gene is located on chromosome 3 in humans. It is expressed mainly in organs like pancreas, liver, kidney and brain[9]. Barroso in 2003 had observed the association of GLUT2 gene with type 2 diabetes due to its role in glucose-induced insulin secretion[10]. Kilpelainen et al., in 2007 stated that risk of type II diabetes mellitus has been associated with common single nucleotide polymorphism (rs5400) of GLUT2 gene, which is commonly formed due to substitution of threonine to isoleucine amino acid at codon 110[11]. GLUT2 gene polymorphism (rs5400) is highly prevalent in Caucasians[12]. Eny et al in 2010 had reported that a variation in individual preferences for sugar containing foods was due to the variations in the GLUT2 gene[13].

The aim of the present study was to evaluate the association of GLUT2 gene polymorphism with sweet perception in dental caries susceptibility in Type II Diabetes mellitus patients.

MATERIALS AND METHODOLOGY

SOURCE OF DATA

This cross-sectional study was conducted in 120 patients with caries aging 30 -70 years who had visited the Department of Conservative dentistry and Endodontics, Rama dental college hospital and research center Kanpur. Ethical approval had been obtained from the ethical committee of the institution and a written consent was obtained from all the subjects participating in the study. All the subjects participating in the study will be divided into two groups.

60 individuals included in GROUP I were known Type II diabetes mellitus patients with caries undergoing treatment for the last five years. Individuals who are diagnosed to be suffering from other diseases were excluded from the study.

60 Individuals included in GROUP II were non-diabetic patients with dental caries. Oral clinical examination was conducted by an examiner who was blinded to the genotype of each individual.

MEASUREMENT OF SWEET PERCEPTION.

All the subjects participating in the study were invited to rate their liking for food which was evaluated by using a 9- point scale ranging from like extremely (score 9) to dislike extremely (score 1). A questionnaire containing a list of 50 items was given to them, and then the mean of liking score given by each individual to these sweet food items was recorded as their sweet liking score which was further used for statistical analysis .

DENTAL CARIES ASSESSMENT

Dental caries of each individual was assessed by using two indexing systems. To conduct accurate oral clinical examination of the participants a dental expert who was blinded to the individual's genotype was assigned to record the occurrence of carious lesions in the oral cavity of each individual, with the aid of an air syringe, mouth mirror and probe. Proximal dental caries and secondary caries were detected by using bitewing radiographs with a horizontal bitewing holder and size 2 films. Using the results of the clinical examination DMFT (decayed, missing, and filled) score was calculated according to the recommendations of WHO for epidemiological surveys. It is applicable for the overall number of teeth present in the mouth. The second indexing system uses a combination of DMFT index with radiographic findings. DMFT + X-ray, was tabulated after combining clinical and radiographic findings. Wisdom teeth were excluded DNA ISOLATION. DNA for the genetic analysis was obtained from the buccal epithelial cells of the buccal swabs for each individual participating in the study. Isolation of DNA was done according to the

manufacturer's using instructions using Puregene buccal cell core kit (Qiagene). Then the amount of DNA was checked with agrose gel electrophoresis. The DNA fragments of cases and the control groups were amplified by PCR (T100 Biorad) to determine the Genotypes of Thr110Ile (rs5400) polymorphism in GLUT2. PCR conditions for GLUT2 gene were 95 ^{0}C for 3 Min, and 37 cycles 95 ^{0}C for 30 sec, 58 ^{0}C for 30 sec, 72 ^{0}C for 1 Min for, and 72^{0}C for 7 Min,12 ^{0}C for forever**.** Restriction endonuclease EcoR1 was used to digest the PCR products. Genotypes **were** determined by fragment size by running 2% agarose gel to check the size of the digested product by RFLP.

STATISTICAL ANALYSIS

Statistical analysis was done using SPSS 21 (IBM Corp., 2012). Standard descriptive statistics were applied in the analysis: mean median and min-max range for quantitative variables. Student's t test was used to compare the sweet likening score, DMFT+X ray , DMFT and its components between diabetic and non-diabetic patients. One way ANOVA was used to compare the sweet likening scores, DMFT + X ray and DMFT with GLUT2 genotypes among diabetic and non-diabetic participants. Tukey's Post-hoc test was done to find out the difference between all possible pairs of GLUT2 genotype with the Sweet likening scores, DMFT and DMFT+X-ray. Statistical significance was set $p<0.05$.

RESULTS:

In the present study 120 adults from the Kanpur population were selected. Out of these 60 individuals were Type II Diabetes mellitus patients with caries and 60 were non-diabetic patients with caries Males (n=61) and females (n=59) were similarly distributed with a mean age 47.48. In the overall sample the mean sweet liking score was 6.58 ±0.73, mean DMFT score was 5.10 ±1.21, and mean DMFT+X ray is 6.01±1.48. Table -1 depicts Student's t test, which was used to compare the mean and standard deviation values of sweet liking, DMFT+X ray; DMFT between the diabetic and non-diabetic patients. Analysis of the data showed over all statistical significance (p=0.001) with higher sweet liking scores (7.195 ± 0.4656), DMFT+X ray (7.25 ± 1.035**)** and DMFT (6.07 ± 0**.**880) scores in diabetic patients when compared with non-diabetic patients. Genetic analysis detected a significant association with Thr110Ile, rs5400, a SNP in the GLUT2 gene. In this study AG showed highest distribution (40.0%) , then AA(35.8%) and GG (24,2%)genotypes. In the Type 2 diabetic population highest distribution was observed in AA (50%), then AG (36%) and GG (11.7%). In non-diabetic patients AG (41.7%)AA genotype (21.7%) and GG (36.7%), In overall participants One way ANOVA is used to compare the sweet likening score, DMFT+X ray and DMFT with GLUT2 genotypes(table 2,3). It was identified that Genotype AA showed significantly higher scores different from their counterparts with regard to their mean score of sweet likening score (7.07 ± 0.57),

DMFT+X ray (6.98 ± 1.43) ,DMFT(5.72 ± 1.09) (p=0.001) and no significant differences were found in this regard between AG and GG genotype. One way ANOVA is used to compare the sweet likening score, DMFT+X ray and DMFT with GLUT2 genotype among diabetic and non-diabetic participants (Table 4). High sweet likening score, DMFT+X ray and DMFT scores were recorded in AA genotype in diabetic individuals than the non-diabetic patients. A positive correlation between sweet liking and DMFT (p value = 0.0001) was observed in this study.

Table 1: Comparison between the sweet likening score, DMFT+X ray , DMFT and its components with diabetic and non-diabetic patients using student's t test.

		Mean	Std. Deviation	Sig.
Sweet likening Score	Diabetic	7.195	.4656	.001*
	Non diabetic	5.970	.3441	.001*
DECAYED	Diabetic	3.15	.685	.001*
	Non diabetic	2.62	.640	.001*
MISSING	Diabetic	.70	.497	.036*
	Non diabetic	.50	.537	.036*
FILLED	Diabetic	2.23	.767	.001*
	Non diabetic	1.03	.450	.001*
DMFT	Diabetic	6.07	.880	.001*
	Non diabetic	4.13	.566	.001*
DMFT+X RAY	Diabetic	7.25	1.035	.001*
	Non diabetic	4.77	.500	.001*

*statistically significant

Table 2**: Comparison between the sweet likening score,** DMFT+X ray and **DMFT** with GLUT2 genotype in overall participants **using** One way ANOVA**.**

	AA (Mean, SD)	AG (Mean, SD)	GG (Mean, SD)	Total	Sig
Sweet likening Score	7.07 ± 0.57	6.55 ± 0.63	5.89 ± 0.53	6.58 ± 0.73	0.001*
DMFT	5.72 ± 1.09	4.90 ± 1.18	4.52 ± 1.05	5.10 ± 1.21	0.001*
DMFT+X-ray	6.98 ± 1.43	5.79 ± 1.23	4.93 ± 0.99	6.01 ± 1.48	0.001*

***statistically significant**

Table 3: **Multiple pair-wise inter group comparison of** SLS, DMFT and DMFT+X-ray **according to GLUT2 genotype using post hoc turkeys' test.**

Variables	Genotype	AA	AG	GG
Sweet likening Score	AA	--	.5270*	1.1825*
	AG	-.5270*	--	.6555*
	GG	-1.1825*	-.6555*	--
DMFT	AA	--	.825*	1.204*
	AG	-.825*	--	.379
	GG	-1.204*	-.379	
DMFT+X-ray	AA	--	1.185*	2.046*
	AG	-1.185*	--	.861*
	GG	-2.046*	-.861*	--

*= The mean difference is significant at the 0.05 level.

Table 4: Comparison between the sweet likening score, DMFT+X ray and DMFT with GLUT2 genotype among diabetic and non-diabetic participants using One way ANOVA.

		AA	AG	GG	Total	Sig.
Diabetic	SLS	7.417	7.039	6.757	7.195	0.001*
	DMFT	6.27	5.83	6.00	6.07	0.193
	DMFTXRAY	7.77	6.83	6.43	7.25	0.001*
Non diabetic	SLS	6.300	6.104	5.623	5.970	0.001*
	DMFT	4.46	4.04	4.05	4.13	0.059
	DMFTXRAY	5.15	4.84	4.45	4.77	0.001*

***statistically significant**

DISCUSSION

Dental caries is one of the mostly prevalent diseases evident in all the age groups of the human population. Preventive strategies of this multifactorial disease include the fluoride exposure, diet management, application of pit and fissure sealants[14]. Diet plays a key role in the occurrence of caries. The present study demonstrated the association of glucose transporter gene polymorphism with the sweet perception and dental caries susceptibility in Type II Diabetes mellitus patients. . Mueckler et al., 1994 stated that the GLUT2 gene is involved in glucose-induced insulin secretion, thus aiding in the regulation of postprandial glucose levels[15]. Physiologically glucose sensing ability of GLUT2 gene in the brain can be attributed to its high Km[16]. In the present study higher sweet liking (7.195 ± 0.4656), DMFT+X ray (7.25 ± 1.035**)** and DMFT (6.07 ± 0**.**880) scores were observed in diabetic patients when compared with non-diabetic patients (p=0.001). Despite of intake of low cariogenic diet the high risk for caries might be due to higher frequency intake of sugary substances and xerostomia, thereby increasing the concentration of glucose in blood and saliva favoring the growth of oral microbiota thus enhancing the high caries risk potential in Type 2 diabetes mellitus[17]. These results are in accordance with the study conducted by Maria Moin and Aeeza Malik in 2015 in which 90% of type 2 diabetic patients had experienced high caries risk[17].

Genetic analysis in the present study detected a significant association with Thr110Ile, rs5400, a SNP in the GLUT2 gene. 50% of AA genotype distribution was observed in Type 2 diabetes mellitus patients then the AG and GG genotypes. Barroso in 2003 observed that increased risk of Type 2 diabetes was associated with Ile allele (AA genotype) in the British population[10]. In the present study high sweet liking scores were recorded in Ile carriers (AA and AG genotypes) in Type 2 diabetic patients. Eny in 2008 reported increased sugar consumption in Ile carriers of GLUT2 gene in adults with early Type 2 diabetes[13]. This further indicates that glucose perception by the GLUT2 gene affecting the glucose homeostasis thus r5400 polymorphism in GLUT2 gene explains the differences in the sugar consumption in humans.

In the present study higher sweet liking score, DMFT and DMFT +X RAY scores in Ile allele (AA genotype) of GLUT2 gene was observed. Statistical significance was observed between DMFT and sweet liking scores thus reflecting a positive association between sweet likings for sweet foods with caries risk. Similarly association of increased caries susceptibility with high sweet liking scores to GLUT2 polymorphism was observed in Italian population[18]. Previous studies revealed that there is no homogeneity in sweet liking in different populations. In this study relationship between diet and caries susceptibility was determined through food questionnaires which served as a simple tool.

Genotype AA showed significantly higher scores different from their counterparts with regard to their mean score of sweet likening score (7.07 ± 0.57), DMFT+X ray (6.98 ± 1.43) ,DMFT(5.72 ± 1.09) (p=0.001) and no significant differences were found in this regard between AG and GG genotype. The results of the present study were in accordance with those reported by Kulkarni in 2012, where they had revealed the association of dental caries with both taste receptor and glucose transporter genes in Caucasian individuals[12]. They had demonstrated significantly high DMFT values in carriers of the Ile variant in GLUT2 gene. Similarly Holla et al in 2015 established the influence of lle allele of rs5400 GLUT2 genes on caries risk in Czech children[19]. This study suggested that, polymorphism of GLUT2 gene contributed to caries prevalence and highlighted the role of sweet liking as a predictor of caries risk.

CONCLUSION

This study concluded that the polymorphism of GLUT2 gene is associated with a higher intake of sugars in Type 2 diabetes mellitus patients. It was also implied that GLUT2 gene polymorphism is responsible for preference for high sugar containing foods thus leading to high risk for dental caries in Type II Diabetes mellitus patients. Further research has to be conducted for confirming the association of GLUT2 gene association in preference for foods and other caries risk factors in various populations of different ethnic back ground.

REFERENCES

1. Featherstone JD, Rodgers BE. The effect of acetic, lactic and other organic acids on the formation of artificial carious lesions. Caries Res. 1981; 15:377-385.
2. Usha Carounanidy and R. Satyanarayanan. Dental caries. A complete change over (PART II) - change over in the diagnosis and prognosis. Jconservative Dent. 2009; 12(3):87-100
3. Featherstone JDB. Dental caries: a dynamic disease process. Australian dental journal.2008;286-291.
4. "Diabetes Fact sheet N°312". WHO. October 2013. Retrieved 25 March2014

5. Williams textbook of endocrinology (12th ed.). Philadelphia: Elsevier/Saunders.1371–1435.arch 48 (1): 44–51.
6. Ramachandran A, Snehalatha C, Kapur A, Vijay V, Mohan V, Das AK, et al. Diabetes Epidemiology Study Group in India (DESI).High prevalence of diabetes and impaired glucose tolerance in India: National Urban Diabetes Survey. Diabetologia. 2001; 44:1094–10101.
7. Malicka B , Kaczmarek U, Zietek M. Dental caries in adult patients with type 1 and type 2 diabetes mellitus. J stoma.2011; 64:9-24.
8. Brown, G. K. Glucose transporters: Structure, function and consequences of deficiency. Journal of Inherited Metabolic Disease, 2000; 23(3): 237-246.
9. Arluison, M., Quignon, M., Nguyen, P., Thorens, B., Leloup, C., & Penicaud, L. Distribution and anatomical localization of the glucose transporter 2 (GLUT2) in the adult rat brain—an immune histo chemical study. Journal of Chemical Neuroanatomy, 2004; 28(3): 117-136.
10. Barroso, I., Luan, J., Middelberg, R. P., Harding, A. H., Franks, P. W., Jakes, R. W., Clayton, D., Schafer, A. J., O'Rahilly, S., and Wareham, N. J.. Candidate gene association study in Type 2 diabetes indicates a role for genes involved in beta-cell function as well as insulin action. PLoS Biol, 2003;1:E20.
11. Kilpelainen, T. O., Lakka, T. A., Laaksonen, D. E., Laukkanen, O., Lindstrom, J., Eriksson, J. G., Valle, T. T., Hamalainen, H., Aunola, S., Ilanne-Parikka, P., Keinanen-Kiukaanniemi, S., Tuomilehto, J., Uusitupa, M., and Laakso, M.. Physical activity modifies the effect of SNPs in the SLC2A2 (GLUT2) and ABCC8 (SUR1) genes on the risk of developing type 2 diabetes. Physiol Genomics. 2007; 31: 264–272
12. Kulkarni GV, Chng Z, Eny KM ,Nielsen D, Wessman C, El-Sohemy a: Association of GLUT2 and TAS1R2 genotypes with risk for dental caries. Caries Res 2013:47: 219-225.
13. Eny KM, Wolever TM, and Fontaine-Bisson B, El-Sohemy A: Genetic variant in the glucose transporter type 2 is associated with higher intakes of sugars in two distinct populations. Physiol Genomics 2008; 33:355–360.
14. J.D.B. Featherstone. The Continuum of Dental Caries— Evidence for a Dynamic Disease Process. Dent Res. 2004;83:(Spec Iss C):C39-C42,
15. Mueckler M, Kruse M, Strube M, Riggs AC, Chiu KC, Permutt MA: A mutation in the GLUT2 glucose transporter gene of a diabetic patient abolishes transport activity. J Biol Chem 1994;269:17765–17767.

16. Manolescu AR, Witkowska K, Kinnaird A, Cessford T, Cheeseman C. Facilitated hexose transporters: new perspectives on form and function. Physiology (Bethesda) 2007; 22: 234–240.
17. Maria Moin and Aeeza Malik. Frequency of dental caries and level of risk among Type II Diabetics. Dentistry 2015; 5:10:1-5.
18. Antonietta R.Lorenzo B, Nicola P , Roberta S, Roberta L, Gasparini P, Ottavia NC. Polymorphisms in sweet taste genes (TAS1R2 and GLUT2) sweet liking and dental caries prevalence in adult Italian population. Genes Nut 2015;10:34
19. Holla. IL, LP Borilova, Lucanova. S, K. Jakub, M Kristina, B. Michaela, K Martina, Klubomir, D Ladisalv. GLUT2 and TAS1R2 polymorphism and susceptibility to dental caries .Caries Res 2015; 49:417-424.