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Frequency of (G428A) Polymorphism within *FUT2* Gene among Symptomatic GIT Diseases in Sudanese Patients

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Abstract:

Digestive disorders and diseases significantly affect millions of persons worldwide inducing a highly significant economical impact comprising health care costs and work absenteeism, in addition to patient's decreased quality of life. Single- nucleotide polymorphisms (SNPs) play a major role in the understanding of the genetic basis of many complex human diseases. Also, the genetics of human phenotype variation could be understood by knowing the functions of these SNPs. This study was carried out in IBNSINA hospital aimed to determine the frequency of the (G428A) polymorphism among Sudanese GIT patients and explore if it has a correlation with type and duration of the disease or not. A total of 49 patients with different GIT infection were enrolled in this study; blood samples were collected from patients and control in EDTA; genomic DNA was extracted from all samples

using salting out method, allele specific PCR used to analyze the samples. The allele specific PCR showed that, 20 (40.8 %) of the 49 patients were homozygous "AA" while that 20 (40.8 %) of the 49 patients were heterozygous (Guanine and Adenine alleles), and just 9 (18.4%) individuals were homozygous "GG". In conclusion, the frequency of the (G428A) polymorphism in the FUT2 gene among the Sudanese patients with GIT disorders was almost the same as the other population, and the correlation between the polymorphism and the duration of the disease has no significant correlation.

Key words: FUT2 gene, GIT diseases, Allele specific PCR, Sudanese

1. INTRODUCTION

Digestive disorders and diseases significantly affect millions of persons worldwide inducing a highly significant economical impact comprising health care costs and work absenteeism, in addition to patient's decreased quality of life [1]. Gastrointestinal tract diseases refer to diseases involving the gastrointestinal tract, namely the esophagus, stomach, small intestine, large intestine, rectum, in addition the accessory organs of digestion, liver, gallbladder, and pancreas. The causes of general GI problems such as constipation, diarrhoea, nausea and vomiting vary widely. Several diseases could be lead to gastrointestinal diseases such as, gastroesophageal reflux disease, oral health considerations and hiatal hernia for the upper digestive tract. Furthermore stomach/intestine disorders, peptic and duodenum ulcers, in addition of cancers and inflammatory diseases which is a common response to viral, parasitic or bacterial infection such as, adenovirus, campylobacter, escherichia coli and helicobacter pylori [2, 3].

Gastrointestinal bleeding is a widespread problem, visible in the form of hematemesis, melena or hematochezia and commonly appears as challenge in primary care centers

and emergency parts [4]. According to United States and United Kingdom annual hospital admittance for GI bleeding, results have been rated at up to 150 patients per 10,0000 populations with a mortality rate of 5-10% [5, 6]. Sometimes GI bleeding is not obvious to the patient and usually presents as positive fecal occult blood or iron deficiency anemia [7]. Accurate clinical diagnosis is crucial for investigation of obscure gastrointestinal bleeding, which is usually from the small intestine [7]. A lot of methods used in diagnosis of GIT diseases, like stool examinations, serological tests, endoscopy which is the most common tool for investigation of GIT problems [8], and recently molecular tests which are generally more sensitive, specific, and rapid than conventional methods [9].

FUT2 gene is a protein coding gene. Fucosyltransferace-2 located on long arm of chromosome 19 at 13.3 and has two exons. This gene is responsible for the secretor phenotype/expression of ABH antigen which constitute molecular basis for ABO blood system which expressed in salivary secretions and gastrointestinal epithelia [10]. This gene is one of two encoding the galactoside 2-L-fucosyltransferase enzyme. Lastly, there are two transcript variants encoding the same protein have been found for this gene [11]. The protein encoded by this gene is a Golgi stack membrane protein that is involved in the creation of a precursor of the H antigen, which is required for the final step in the soluble A and B antigens synthesis pathway [11]. Protein attributes for *FUT2* gene consist of, 343 amino acids and 39017 Da (Dalton) molecular mass [12].

ABH secretion is controlled by two alleles Se and se. Previous studies around the world showed, approximately 60-80% of people are secretor (SeSe or Sese), and furthermore blood group "O" individuals (males and females) scored the highest frequencies of secretor status among different ABO blood groups [13-15]. By the same token, studies showed the

relation between secretor status and GI diseases such as, norovirus infection, H.pylori infection and gastroenteritis [16-18].

Single nucleotide polymorphisms (SNPs) are variations of a single base, either between two homologous chromosomes within a single individual, or between two individuals. Genetic polymorphisms are well-recognized sources of individual differences in disease risk and treatment response [19]. SNPs are found throughout the genome in exons, introns, intergenic regions, promoters, enhancers, SNP in a promoter can influence gene expression [20].etc and thus more likely to contain an allele being more functionally or physiological relevant than other types of polymorphism. There are about 500,000 SNPs fall in the coding regions of the human genome [21]. Among these SNPs, nonsynonymous SNPs (nsSNPs), those cause changes in the amino acid residues. Missense, nonsense and frameshift; are the nsSNPs types. These are likely to be an important factor contributing to the functional diversity of the encoded proteins in the human population. Nonsynonymous SNPs affect the functional roles of proteins in the signal transduction of visual, hormonal, and other stimulants [22]. Identification of SNPs responsible for specific phenotypes seems to be a problem, since requiring multiple testing of hundreds or thousands of SNPs in candidate genes.

The aim of the present study was to determine the frequency of the most common polymorphism (428 G-A) within *FUT2* gene among symptomatic GIT Sudanese patients.

2. MATERIAL AND METHODS

This study is a descriptive cross sectional study, conducted in Khartoum state, to determine the frequency of (428 G-A) polymorphism within *FUT2* gene among symptomatic GIT Sudanese patients attending IBNSINA hospital during the

period of August 2015 to May 2016. 49 Samples collected from diagnosed symptomatic GIT patients from different ethnic groups.

EDTA blood sample (3ml) was collected from each, and then DNA was extracted using salting out method. For all samples genotyping was performed using allele specific polymerase chain reaction (PCR-TECHNE TC412, UK). Each PCR tube of 20 µl contains, 3 µl of genomic DNA, 5 µl of master mix (maximum PCR premix kit (i-taq) iNtRON, Korea), 1 µl of antisense primer (5-GGCTGCCTCTGGCTTAAAG), 1 µl to one of reference allele (5-GCTACCCCTGCTCCTGG) or variant allele (5 CGGCTACCCCTGCTCCTA), and 10 µl of water (distilled water).The amplification process were as follow, denaturation at 95°C for 3 minutes, annealing at 54°C for 1 minute, and extension at 72°Cfor 5 minutes. Then PCR products were placed in electrophoresis. 3 ul of 100 Pb (base pair) DNA ladder was applied with each batch of patient samples. Data was collected by structured questionnaire and analyzed using SPSS software version 21.

This study was approved by IBNSENA and faculty of medical laboratory sciences, Alneelain University as well as consent was taken from patients.

RESULTS

The study included 49 GIT patients; 32 (65.3%) of them were males and 17 (34.7%) were females, the age ranged between 30-89 years (mean 51.4, SD 16.4).

The allele specific PCR showed that, 20 (40.8 %) of the 49 patients were homozygous (the two alleles were Adenine), while that 20 (40.8 %) of the 49 patients were heterozygous (Guanine and Adenine alleles), and just 9 (18.4%) individuals were homozygous "GG" (**Table 1**).

Table 1: the frequency of (G-A 428) FUT2 polymorphism among the study group.

Diagnosis	Frequency	Percent
Homozygous "GG"	9	18.4
Heterozygous	20	40.8
Homozygous "AA"	20	40.8
Total	49	100.0

There was no significant correlation between the type of GIT infection and the polymorphism detected the as well as the duration of the disease with (P.Value= .856, 0.124) respectively. The Genotyping of the FUT2428 showed an increased (AA) among male comparing to females (Table 2).

Table 2. Distribution of genotyping among gender

Gender	Homozygous "GG"	Heterozygous	Homozygous "AA"	Total
Male	3	15	14	32
Female	6	5	6	17

In Table 3 the genotype in each age group demonstrate that the age does not affect the hetro or homogeneity of the sample.

Table 3: Distribution of genotyping among age

Age	Homozygous "GG"	Heterozygous	Homozygous "AA"
30.00 - 44.00	8	5	7
45.00 - 59.00	1	7	6
60.00 - 74.00	0	3	6
75.00+	0	5	1

DISCUSSION

Gastro intestinal tract disease is common problem in Sudan and recently there is increasing in incidence of stomach ulceration, pancreatitis, and gastritis.

This study conducted to determine the frequency of the FUT2 (428 G-A) polymorphism Sudanese patients with GIT infections, the allele specific PCR results showed that 20 (40.8

%) was homozygous, 20 (40.8 %) was heterozygous while 9 (18.4%) are normal among gastrointestinal infection patient, these results are support the findings of previous study done by (Muddathir, A.M. et al.) which explained in their study the phenotype prevalence rate for normal (SeSe), heterozygous (Sese) and homozygous (sese) were 68(12%), 112(19.8%) and 386(68.2%) respectively [27].

Age ranged from 30 to 89 years, the result showed there was an increase susceptibility of the 428 G-A in the FUT2 gene polymorphism with decreasing age but observationally without statistical correlation, These demonstrate that there was no significant correlation between age of Sudanese's patients and FUT2 gene polymorphism. The present study demonstrated that there were an increased number of males compared to females.

This study also showed that 9 (18.4%) are genotype (AA) While 40(81.6%) (GG OR GA) among gastrointestinal infection patient, our findings can be compared with those of (Wacklin et al. 2014) which showed that the microbiota association with the secretor status and *FUT2* genotype was studied in more detail for the subset of 24 samples (12 non-secretors, AA genotype and 12 secretors of which 5 carried genotype GG and 7 carried genotype AG) by pyrosequencing [28].

Individuals with secretor phenotype were either homozygous (GG) or heterozygous (GA) while the AA genotype considered non secretor as study done by (Wacklin, P., Mäkivuokko 2011) [29].

Our result similar to (Maroni et al. 2015) that showed the Fucosyltransferase 2 (FUT2) mediates the inclusion of fucose in sugar moieties of glycoproteins and glycolipids. ABO blood group antigens and host-microbe interactions are influenced by FUT2 activity. About 20 % of his population has a "non-secretor" status caused by inactivating variants of FUT2 on both alleles [30].

The non-sense mutation G428A and the missense mutation A385T are responsible for the vast majority of the non-secretor status in Caucasians, Africans, and Asians, respectively. The distributions of nonsense mutation (428 G-A) in the *FUT2* gene which is the most frequent polymorphism.

In addition, many studies showed the relation between *FUT2* gene polymorphisms and disturbed in ABH secretion in conjunction that lead to large number of diseases.

CONCLUSION

In conclusion, frequency of the FUT2 gene polymorphism among gastrointestinal infection was significantly high than the control grouped. Large percentage of Sudanese individuals has got an "AA" genotype allele in FUT2 gene position 428; this large percentage could be according to human genomes variations worldwide.

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