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DISTRIBUTION OF SOME ALLELES IN HUMAN DNASE 1 GENE FOR A SAMPLE OF IRAQI POPULATION

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ABSTRACT
The deoxyribonuclease 1, designated as DNASE1 gene in which it has, single nucleotide polymorphisms (SNPs), considers as a valuable marker in clinical investigation and genetic. Genotyping of the DNASE1 alleles polymorphisms within the DNASE 1 gene was performed in a sample of Iraqi population. In this study, the identification of the fourth alleles of DNASE1 gene using polymerase chain reaction .The results clarified the distribution of the four alleles of DNASE1 gene and demonstrates that there is a certain genetic heterogeneity in the samples, Furthermore, the DNASEl*1 allele was found to be the most predominant (65%) among the samples (100%), with molecular base (261 bps), 430 bps for DNASE1*3, and 139bp for DNASE1*2 and *4.. The DNASE l*4 allele can be distinguished from the DNASE l*2 allele by PCR-RFLP based on a G-C transversion in exon 1 of the gene. The result showed that seven samples(7%) among twenty two (22%)of human DNA appeared positive result when digested with *XhoI* enzyme with a band of116 bp which is related to DNASE1*4 ,while fifteen samples showed negative result with a band of 139 bp which is related to DNASE1*2.

KEY WORDS: DNASE1 gene, deoxyribonuclease I *alleles*, single nucleotide polymorphism, populations.

INTRODUCTION

Nucleases considered as important analytical enzymes which are used widely in biotechnological and biomedical application; this application depends on the mode of action and specificity of a particular enzyme. Nucleases had been employed in the production of flavor enhancers, removal of nucleic acids, as therapeutic agents, and for the determination of nucleic acid structure, in particular, mutation typing[1]. Endogenous deoxyribonuclease I (DNase I, EC3.1.21.1) have been noted as candidate endonucleases involved in the breakdown of chromatin during necrosis and apoptosis[2]. Deoxyribonuclease I has been highlighted for its possible involvement in the pathogenesis of systemic lupus erythematosus (SLE)[3] .Others human genes encoding types of DNase, potentially relevant to autoimmune diseases[4]. Dysfunction of DNase I* 2 play a role in the etiology of parakeratosis through incomplete degradation of DNA in the epidermis[5]. The applied this nuclease to single nucleotide polymorphism (SNP) typing. SNP detection has considerable significance of association studies of complex diseases[6], pharmaco genetics[7], population genetics[8], and physical mapping [6]. A number of different methods have been reported to the detection of single nucleotide variations [9,10]. Within a population, SNPs can be assigned the lowest allele frequency at a locus that is observed in a particular population. There are variations within different human populations, hence a SNP allele is common in one ethnic group may be much rarer in another [11]. Members of the human DNASE I family, with physiological role(s) other than those of DNASE I, possess three and one non synonymous SNPs in the genes, respectively. However, only limited population data are available, and the effect of these SNPs on the catalytic activity of the enzyme remains unknown[12]. Within the human DNase family genes, it seems that *DNASE1* is able to tolerate the generation of nonsynonymous SNPs, and that the amino-acid substitutions resulting from the SNPs in DNASE1 easily alter the activity[3].The gene encoding human DNase 1*1 is located in region q28 on the X chromosome ,the long of DNase I gene was approximately 3.2 kilobases, it comprises nine exons with eight introns separated them [13,14] .The single-nucleotide polymorphisms (SNPs) in the human DNase I gene (DNASE1) might be involved in susceptibility to some common diseases; however, only limited population data are available. Further, the effects of these SNPs on in vivo DNase I activity remain unknown [15].Genotyping of all the non-synonymous SNPs was performed in three ethnic groups including six different populations using the PCR-RFLP method newly developed, Asian and African groups including Japanese, Koreans, Ghanaians and Ovambos were typed as a single genotype at each SNP, but polymorphism at only SNP V122I in DNASE 1 was found in Caucasian groups including Germans and Turks; thus a Caucasian-specific allele was identified[16]. To accumulate data on the genetic distributions of SNPs in many populations derived from different ethnic groups, it is still unclear whether individual SNPs in *DNASE1*1* may serve as a functional SNP affecting its *in vivo* activity of the enzyme [17].The aim of this study was to describe the molecular analysis for four alleles, and investigated the prevalence of these alleles in a sample of Iraqi population.

MATERIALS & METHODS

Sample Collection

The one hundred human blood samples were collected from different regions in Baghdad governorate (South,

west, east) included different locations, gender of male and female with ages ranged between (18-45) years old.

DNA Extraction

The genomic DNA was extracted from blood samples using nucleic acid extraction kit (promega mini kit, USA). The amount of total DNA extracted from each sample was measured by Nanodrop D-1000 (Thermo scientific, USA) to determine the extraction DNA concentration and purity. The DNA extraction was detected using agarose gel electrophoresis according to a method described by [18].

Molecular Analysis of genomic DNA using- PCR technique

Specific sequence decamer primer was used in this study synthesized by (promega\USA) from different series OP (Operon Technologies according to[19], the lyophilized form of specific primer were dissolved in sterile deionizer distilled water in final concentration (10pmol/µl) . Table (1) showed the list of specific primers. PCR mix prepares as recommended by providers, to achieve a total volume of 25 µl of the final reaction volume the preparation was as follows: Aliquot of 12.5µl of the Master Mix 2X (Taq®Green Master Mix/Promega-USA) in final concentration (1x), sterile distilled water (10µl), each primer reverse and forward (0.5 µl) and DNA template (1.5µl). The PCR program was preformed as follows: Initial denaturation for 5 min at 94°C, second denaturation for 1 min at 94°C, Annealing at (58,60,62,66) °C for (30,45,60) sec, extension for 1 min at 72°C and final extension for 10min at 72°C. The cycle numbers was 35. A volume of 5µl (amplified products) was detected by electrophoresis in 2% agarose gels (5V/cm, 1 hrs, 0.5X Tris-borate buffer). The gel was stained with ethidium bromide; and the PCR products were visualized by U.V transilluminator and then were imaged by gel documentation system, to estimate the size of PCR product the DNA Ladder (100 bp) was used as a marker.

TABLE 1: The sequence of the specific primers

No.	Primers name	Sequence 5----------3
1-U-1	Foreword primer(**F**)	**'ATCGTGGTTGCAGGGATGCTGCCTC**
D-1	Reverse primer (**R**)	**AGTTCAACAGGTGTGGGGAG**
2 - U-2	Foreword primer(**F**)	**GTCAGGGAGTTTGCCATTGTTG**
D-2	Reverse primer(**R**)	**'AAGGCTTTGAGGCTTCTGAA**
3 - U-3	Foreword primer(**F**)	**'CCTGAAGATCGCTGCCTTCAACTC**
D-3	Reverse primer (**R**)	**'ACCAGCCCTAGACTCCAGAG**

Genetic Polymorphism of DNASE1*4 and DNASE1*2 using *PCR - RFLP*

To detect the *DNASE1-4* allele from *DNASE1-2* allele, the mismatched PCR method,was employed . The sequence that included the nucleotide substitution which generated the mutation was amplified by the using primers,

U-3(5_-**'CCTGAAGATCGCTGCCTTCAACTC**-3_)

D-3 (5-**ACCAGCCCTAGACTCCAGAG** -3).

In order to create a new *Xhol* enzyme site at the mutation site in the *DNASE1-4* allele, following amplification, the PCR product was digested with *Xhol*. The preparation buffer for RFLP was carried out as the following: Aliguot (2µl) of the Buffer mix (bio lab/ 10X) was added to achieve a final concentration (1X) ,sterile distilled water (9µl), 4µl of enzyme(10u) to obtain a total volume of 20µl after addition 5µl of PCR product .The tubes of RFLP mixture incubated in water bath at 37c for 2h. After the incubation, 10µl of RFLP mixed products was separated in 3% agarose by electrophoresis (1hour, 5Voltage /cm , 1Xfrom TBE buffer). The gel was stained with ethidium bromide; the result of products (PCR and RFLP) was visualized by U.V transilluminator and then was imaged by gel documentation system. The amplified product usually consists of 1discrete bands ,and the RFLP product consists of 2 bands ;the size of RFLP product estimated by comparing with the PCR amplified and the marker; DNA Ladder (100 bp).

RESULTS

Sample collection

The one hundred human blood samples were collected randomly from healthy Iraqi individuals (male and female with a mean age of 31.5 ±13.5 years). The genomic DNA was extracted efficiently using nucleic acid extraction kit, regarding purity and concentration. The yield of the DNA extracted from human blood was in the range of (171-2267) ng /µl with a purity of (1.7-2).

Polymerase chain reaction (PCR) amplification for Deoxyribonuclease I gene

The DNASE1*1,*2,*3and *4 alleles were identified using PCR technique with specific primers. The results showed that the specific primers used to investigate the present of this gene *DNASE1*- alleles in DNA of one hundred human blood samples exhibited the amplified primer that appeared as bands in all blood samples (Figure-1,Figure-2 and Figure-3). In order to estimate the approximate size of the bands after being amplified and scored on the agarose gel electrophoresis, the results revealed that the optimum annealing temperature was 62ºC for 45 sec, the specific primer (U1) and (D1) used to investigate the present of the *DNASE1**1 and *DNASE1**3 alleles in DNA of human blood samples. The results in figure (1) exhibited positive results in seventy eight blood human samples, with a band of approximated 261 bps compared with a ladder (100bp) in addition to a negative control (no band). According to the results revealed it was necessary to distinguish between the *DNASE1**1allele from *DNASE1**3 allele, hence another substitution nucleotide was used in the primers U2 and D2 as shown in table (1) which is specific for *DNASE1**3 allele from other alleles *DNASE1**1, *DNASE1**2 and *DNASE1**4 .The results in figure (2), appeared single DNA band with a molecular weight of 430bp due to the presence of the *DNASE1**3 allele in DNA of thirteen human blood, On the other hand the results of *DNASE1**2 and *DNASE1**4 alleles appeared in a single band with a molecular weight of 139bp in twenty two samples (figure 3). **So** the results revealed that the nucleotide sequence of *DNASE1**4 allele was identical to

that of *DNASE1**2 allele except for a single nucleotide at position 91 and to that of *DNASE1**1 allele except for two nucleotide at position 91 and 2317.

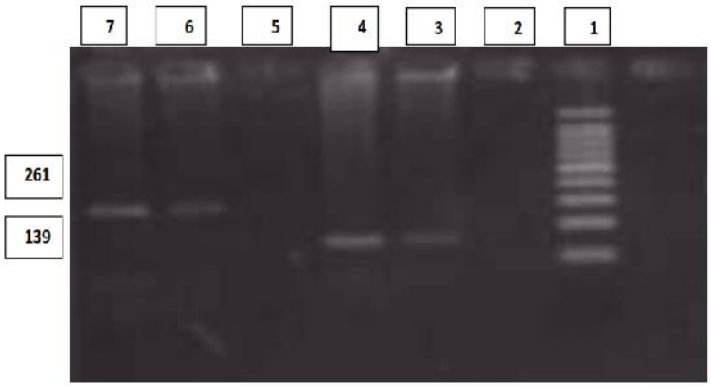

FIGURE 1: Agarose gel electrophoresis of PCR product for Deoxyribonuclease I gene fragment amplified using specific primers by electrophoresis on 2% agarose gel, 5 (Vol/cm), (1.5hr) in 1x TBE. Lane 1 M - 100 bp DNA ladder, lane 2, negative control. lane 3,4, : 139bp PCR product of Deoxyribonuclease1 *2 ,*4,lane 6 ,7: 261bp PCR product of Deoxyribonuclease1 *1 allele

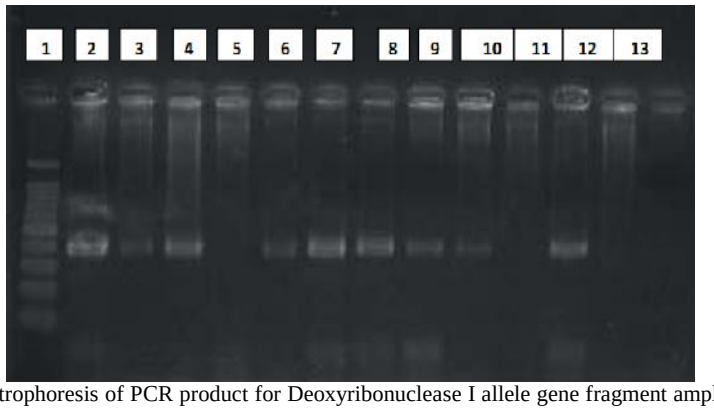

FIGURE 2: Agarose gel electrophoresis of PCR product for Deoxyribonuclease I allele gene fragment amplified using specific primers by electrophoresis on 2% agarose gel, 5 (Vol/cm), (1.5hr) in 1x TBE buffer. Lane 1, M - 100 bp DNA ladder- lane 5. 11,13, negative result- lane (2,3,4 6,7,8,9,10,12) : 430 bp PCR product of Deoxyribonuclease1 *3.

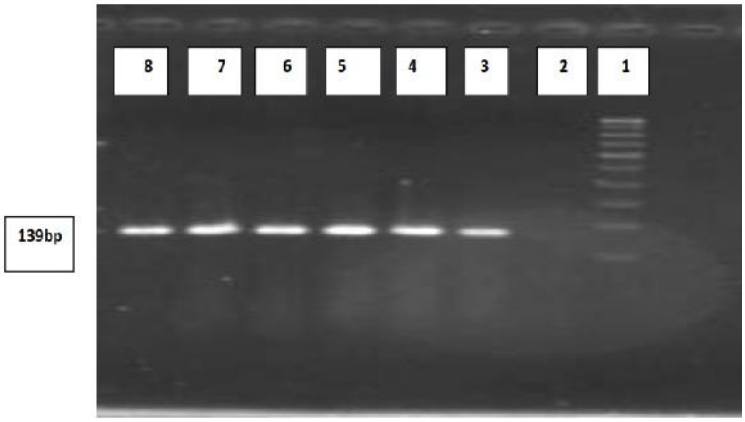

FIGURE 3: Agarose gel electrophoresis of 139bp PCR product for Deoxyribonuclease I gene fragment amplified using specific primers by electrophoresis on 2% agarose gel, 5 (Vol/cm), (1.5hr) in TBE buffer and visualized by ethidium bromide staining under ultra transilluminator UV. Lane 1,M - 100 bp DNA ladder lane 2, negative control. lane 3,4,5,6,7,8,: 139bp PCR product of Deoxyribonuclease1 *2,*4. .

Genotyping of DNA samples using *PCR - RFLP*

The result of this study showed that seven samples of human DNA *appeared positive when digested with XhoI* enzyme, *while fifteen (0.15) showed negative results (not digested with XhoI* enzyme) (Figure-4). This result revealed one non-synonymous SNP in the gene however, only limited population data are available from total samples.

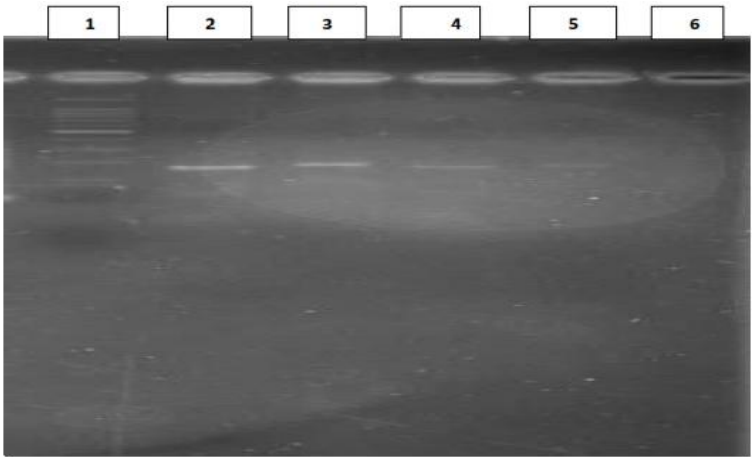

FIGURE 4: The electrophoresis of digested PCR products amplified from genomic DNA by *Xha1* enzyme, and non digestive PCR products. on 3% agarose gel, 5 (Vol/cm), (1.5hr) in 1xTBE buffer. Lane 1, M- 100 bp DNA ladder, lane 2, 4: 116bp digested PCR products. lane3, 5: 139bp PCRproduct.

DISCUSSION

At this study, it was performed that the genotype distribution for four SNPs in the DNASE1 gene (four alleles) (Figure-1,2 and 3) included population from Baghdad government .The results had been provided data for these SNP sites in sample of Iraqi population. Furthermore, it was clarified the distribution of the four alleles of DNASE1 gene. However, rather low frequency (7.0%) for allele 4, 13% for allele3, while 15% for allele 2,but high frequency for allele 1.Generally, the DNASE 1 gene show diversity for genetic structure to the SNPs. The A2317G in exon 8 was amplified by PCR using a set of D1 and U1 primers which in appeared 261 bp DNA fragmen(figure-1).The difference between DNASE*1 (DNASE*3) at the polymorphic site A2317, and DNASE *2(DNASE*4) at G2317 is due to an A-G transition at the position 2317 in exon 8, this variation in SNPs lead to substitution at amino acid Gln to Arg at position 222 of DNase I[20]. Therefore the results of the different polymorphic site in the position of the substitution nucleotide in the primers (Table-2), it identifies the allele appeared in the template of DNA. The results at the figure (2) showed the present of DNASE1*3 allele in thirteen samples from the seventy-eight samples of human DNA that appeared positive result for DNASE1*1 using specific primers U2 and D2 to discrimination the type 3 alleles. This explains the results of one study by [20] which noticed the third allele DNASE1*3 that have one nucleotide substitution, a C-G transition (CCC-->GCC), in the codon for amino acid 132 of the mature enzyme located in exon VI was found as a result of replacement of proline with alanine (P132A) while the other 3 alleles DNASE1*1, DNASE1*2and DNASE1*4 have C in the same position. The results of this study revealed that the nucleotide sequence of *DNASE1*4 allele was identical to that of *DNASE1*2 allele except for a single nucleotide at position 91 and to that of *DNASE1*1 allele except for two nucleotide at position 91 and 2317. [19] . All samples of DNA that possesses *DNASE 1* *2 allele and *DNASE 1* *4 allele *alleles* (figure 3), was detected by PCR-RFLP to confirm the SNP site, the method of mismatched PCR was used. The exon 1 sequences of the mutation site were amplified with a pair of primers, the sequences of which were: 5'-CCTGAAGA TCGCAGCCTTCAACCTC-3' (sense primer) and 5''-ACC AGCCCTAGACTCGAGAG-3' (antisense primer). This means that the PCR product amplified from the fourth allele *DNASEI*4* would have a newly created *XhoI* site (CTCGAG) at the mutation site, whereas that from the other alleles would not contain this site. *The XhoI* enzyme was considered as DNA restriction enzymes (from *Xanthomonas holcicola)* which cut the sequence in specific site.

C | TCGA G
G AGCT | C

The substitution nucleotide corresponding to SNP would be discriminating factor of polymorphism in *Xhol* digestion. PCR products were used in restriction enzyme (*Xhol*) digestion. Two different DNA bands on gel electrophoresis were expected depending on the SNP. A sample genotype with C nucleotide (C allele) in *Xhol* recognition site cannot be digested by the enzyme and the molecular weight of DNA band is 139bp and this result revealed to the two type alleles of *DNASE 1* gene (*DNASE1* *2 and *DNASE1* *4). Whereas a SNP with G nucleotide (G Allele) instead of C, will be a target of *Xhol* restriction enzyme. The digested DNA band is 116bp and this result confirmed *DNASE 1* *4 allele. The results showed that seven samples were digested with *Xhol* enzyme related to *DNASE 1* *4 allele, while fifteen was related to *DNASE 1* *2 allele. In other words seven samples have G allele and fifteen has C allele nucleotide. This result revealed to a mutation encoded by a fourth allele, *DNASEI*4*, was detected and distinguished from allele two, *DNASEI*2*, by PCR-RFLP using restriction enzyme *Xhol*. DNASE I is an endonuclease that attacks preferentially double-stranded DNA in a dependent manner (Ca2+) to produce oligonucleotides with the 5¢ phosphor and the3¢-hydroxyl termini[21], DNase I is an enzyme that encoded by a single gene DNASE1, which was located on chromosome 16 within the p13.3 region[19] . Fujihara and his colleagues, revealed the existence of a certain genetic heterogeneity in the world and the distribution of DNase I gene polymorphisms[23]. The results of the PCR-amplified fragment using SSCP

analysis reported that 81 German individual's, and 114 unrelated Japanese observed variant in a Japanese man. The other previous study obtained by [16] of three groups (ethnic groups) including six different populations , African and Asian groups including Japanese, Ghanaians, Koreans and Ovambos were typed at each SNP as a single genotype, but polymorphism at only the SNP V122I in DNASE 1 was found in other Caucasian groups including Turks and Germans. These findings postulates the non-synonymous SNPs identified with the DNASE 1 gene ~~may~~ exert no influence on the activity levels of DNASE 1L1 in human populations. Other study investigated the distribution of DNASE 1*1 and DNASE 1*3 in exons of the gene in three Caucasian and African, in the eight Asian populations worldwide using newly devised methods genotyping [24]. almost all of the non-synonymous SNPs in the genes encoding other members of the human DNASE family, DNASEI*1, I*2, and 1*3, exhibited a mono-allelic distribution of the same study populations, similarly to those of DNASE I*1 and I*3 [25].The extremely high frequencies of DNASE1*1 in the population from two African populations Ghanaian was 0.90 and Xhosa was 0.88. and this result is similar to that population of the Ovambos living in Namibia comparaisms with other study for Caucasians and Asians population had a lower frequency for DNASE1*1 than the African groups[26]. Several non-synonymous SNPs in the human DNASE1*2 responsible for DNA degradation during terminal differentiation of epidermal keratinocytes have been identified. However, only limited population data are available, and furthermore the effect of these SNPs on the DNASE1*2 activity remains unknown[23].In this study which considered the first to detect the polymorphisms of DNASE1gene in different alleles in one hundred DNA sample using PCR and PCR-RFLP mismatched method, represents the existence of DNase 1 polymorphism in Iraqi population. The other alleles appeared to be limited among Iraqi people except DNASE1*1 which was dominant. In conclusion, this study gives a picture of the genetic diversity of DNASE 1gene alleles in a sample of Iraqi population.

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