Study of Some STR Markers of Y – Chromosomes in Two Iraqi Sub – Populations of Middle – Euphrates Aria

Mohammed zuhair Naji[1], Ali Hmood Al-Saadi[2]

Mohammed zuhair Naji[1], Ali Hmood Al-Saadi[2]
[1]Ph.D. student in biology department of science in University of Babylon, Babylon, Iraq.
[2]prophesier in biology department of science in University of Babylon, Babylon, Iraq.

Correspondence:
Mohammed Zuhair Naji
Ph.D. Student in Biology Department of Science in University of Babylon, Babylon, Iraq
E-mail: biomanearthlink@gmail.com

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ABSTRACT

In this study, a sample of 144 persons lived in middle-Euphrates of Iraq population was analyzed using 5 Y-chromosome short tandem repeat (STR) polymorphisms. This population (n=144) was separated to two sub-population Hashemites (n=72) and Commoners (Non- Hashemites) sub-population (n=72), in the commoners sub-population the locus DYS19 was most polymorphic loci in number of alleles with the highest gene diversity, while in Hashemites sub-population, the locus DYS19 and DYS392 were most polymorphic loci in number of alleles and DYS391has the highest gene diversity. In study of Y STR Haplotypes, it found 62 haplotypes in all populations 52 were unique haplotypes, the Hashemites sub-population the number of haplotype was 27, the unique haplotypes were 19, and in the commoners sub-population the number of haplotype was 35, the unique haplotypes were 33 haplotypes and has a highest haplotype diversity and Discrimination Capacity.

Keywords: Y STR, Hashemites, Haplotype diversity, Discrimination Capacity

INTRODUCTION

Depending on the origins of the Iraqi Arab tribes, it can be divided broadly into two parts: Hashemites clans, whose origins go back to Bani Hashem, the grandfather of the Prophet Muhammad and general families (non-Hashemites) (Curatola, 2007).

The Y chromosome is a sex- determining chromosome. The Y chromosome is one of two sex chromosomes which called allosomes. Traits that are inherited via the Y chromosome are called Y linked traits "holandric traits" (Turner *et al*., 2004). Y chromosome contain the Sex-determining Region Y gene (SRY) which encode a protein that triggers testes development (Goodwin *et al*., 2011). The Y chromosome is one of the smallest human chromosomes, with an estimated average size of 60 million base pairs. During male meiosis recombination only takes place in the pseudoautosomal regions at the tips of both arms of Y and X chromosomes (PAR1, with 2.6 Mb, and PAR 2, with 0.32Mb). Along _95% of its length the Y chromosome is male-specific and effectively haploid, since it is exempt from meiotic recombination. Therefore, this Y-chromosome segment where X-Y crossing over is absent has been designated as the non-recombining region of the Y chromosome or NRY. Because of the high non-homologous recombination occurring within this Y chromosome specific region, a more appropriately name of male-specific region or MSY is nowadays used to designate it (Skaletsky *et al*., 2003).

The Y chromosome is specific to the male portion of a male-female DNA mixed sample such as vaginal swap which is commonly seen in sexual assault cases (Park *et al*., 2012).

Microsatellites these refer to DNA with varying numbers of short tandem repeats (Allor *et al*., 2005; Klintschar *et al*., 2006) between a unique sequence DNA regions with repeat units that are 2 bp to 7 bp in length or most generally short tandem repeats (STRs) or simple sequence repeats (SSRs) are generally known as microsatellites (Ellegren, 2004).

The Y chromosome, excluding the pseudoautosomal region, is transmitted down the paternal line from generation to generation with few modifications due to mutation and gene conversion (Rozen *et al*. 2003; Trombetta *et al*. 2010). These inheritance properties have encouraged the use of genetic polymorphisms on the Y chromosome in several areas of research. Short tandem repeat (STR) polymorphisms are commonly used in population evolutionary genetic studies, providing an alternative or complement to the single nucleotide polymorphism approach (Jobling and Tyler-Smith 2000). In fact, Y-STR haplotypes were found to be informative in discerning recent events of human migration (Pereira *et al*. 2002). Roewer *et al*. (2005) recommended that, notwithstanding the uncertainty in ascertaining a recent or prehistoric origin for a genetic pattern on the basis of Y-STR analyses alone, these markers should be the choice for studies on local population structure and recent demographic history.

MATERIALS AND METHODS

Collection samples

Samples have been collected from 144 person, they separated in two groups: Hashemites and, Commoners (72 persons for each). These samples was collect from middle Euphrates aria (Baghadad, Hillah, Karbala, Najaf, Kut and Diwaniyah), the work done in the Biotechnology and Genetic Engineering Laboratory, Department of Biology, College of Science, University of Babylon.

DNA extraction methods

Genomic DNA of blood were extract using DNA extraction kit (FAVORGEN)

Amplification Y-chromosome STR

Five Y STR marker (Dys19, Dys390, Dys391, Dys392, Dys393) were use in this study on Iraqi population. PCR amplification was done using conventional thermocycler (Biometra - Germany)

Agarose Gel Electrophoresis

The agarose gel electrophoresis was perfomed according to the method of Robinson and Lafleche (2000). This technique was used to detect genomic DNA extracts and PCR products.

Cycle sequencing and Sequence Analysis

The DNA Sequencing of the PCR products was done using the BigDye TM Terminator (reaction mixture consisted of: 5 uL purified products, 2 uL Big Dye Terminator, 2 ul. sequencing buffer and 1 uL reverse primer) Utilizing POP-7 polymer (Applied Biosystems) of lot number 1206453. The separation of the cycle sequencing products and detection were carried out by using the ABI 3730XL DNA Analyzer, cap array size 96, cap array length 50. The reference sequence described by (Anderson, 1981) was compared to the data observed.

Statistical Analysis for Y- Chromosomal STR

Allele frequencies

After the samples have been collected, extracted DNA and amplified PCR were genotyped for the 5 Y STR loci of interest. The genotyping information was then converted into allele frequencies by Relative allele frequencies for each population and the overall population were calculated by dividing the number of occurrence for each allele by the sample population size (Butler *et al*., 2002). Allele frequencies for Y- Chromosomal STR were calculated by direct counting, therefore:

Allele frequencies = Total no. of alleles / Total no. of samples.

Allele diversity (Genetic Diversity)

Is the actual number of alleles present at a locus. Allele diversity was calculated as (Nei, 1987).

$GD = N * (1-\sum X2) / N-1$

Where N is the sample size and X is the frequency of the allele

Haplotype frequency

Haplotype frequency it means of each haplotype of the sample found with any sample size. Haplotype Frequency was calculated by using the Excel program.

Gene diversity (GD)

GD was calculated for each Y - STR according to the formula supplied by Nei (1987) and Gusmao *et al*. (2006): D - N - (1 - 2) | Where is the same Size and the relative allele frequency. Gene diversity among Populations occurs there are differences in allel frequencies between those populations, |

Haplotype diversity (HD)

HD was calculated using the same equation as calculating gene diversity using haplotype frequencies instead of allele frequencies (Gusmao *et al*., 2006)

Discrimination Capacity (DC)

DC was calculated using the following formula (Gusmao *et al*., 2006)

Discrimination Capacity = Number of unique hap. / Number of hap.

RESULTS AND DISCUSSION

Y-STR Allele frequency and Genetic Diversity for Hashemites sub-population (n=72)

The allelic frequencies of 72 healthy male individuals involving five Y-STR loci, which analyzed for diversity of Hashemite sub-population (table 1) show the loci DYS392 and DYS19 were most polymorphic with six number of alleles , while loci 390 and 393 were less polymorphic with four number of alleles , and locus 391 has 5 number of alleles.

The allele no. 12 of locus DYS393 has a highest frequency (0.458), but allele no. 15 of locus DYS392 and allele number 11 of locus DYS393 has a lowest frequency (0.028).

The lowest gene diversity (0.605) has been found in locus DYS393, But the highest gene diversity (0.787) has been found in locus DYS391, the reminds gene diversity (0.690, 0. 747, 0.784) return to DYS390, DYS392 and DYS19 respectively.

The distribution of allele frequency of each Y STR loci in Hashemites sub-population was show in Figure (1), locus DYS390 has four alleles (23, 24, 25 and 26) , allele No. 25 has a highest frequency (0.389) and allele No. 26 has a lowest frequency (0.125). Locus DYS391 has five alleles (9, 10, 11, 12 and 13), allele No. 10 has a highest frequency (0.278) and allele No. 13 has a lowest frequency (0.083). Locus DYS392 record six alleles (10, 11, 12, 13, 14 and 15), allele No. 12 has a highest frequency (0. 347) and alleles No. 15 have a lowest frequency (0.028). Other Y STR locus DYS393 has four number of alleles (11, 12, 13, 14) allele No. 12 has a highest frequency (0.458) and allele No. 11 has a lowest frequency (0.028). Finally, locus DYS19 has six number of alleles (12, 13, 14, 15, 16 and 17) allele No. 13 has a highest frequency (0.555) and allele No. 15 and 16 have a lowest frequency (0.056).

The allelic frequencies of 72 healthy male individuals involving five Y-STR loci, which analyzed for diversity of Commoners sub-population as show (table 2).

The locus DYS19 was most polymorphic loci with six number of alleles, while remind loci DYS390, DYS391, DYS393 were less polymorphic with four number of alleles and locus DYS392 has five number of alleles.

The allele no. 11 of locus DYS393 has a lowest frequency (0.028), but allele no. 13 of locus DYS393 has a highest frequency (0.486).

Gene diversity values for each Y-STR loci have been given in Table (3). The lowest gene diversity (0.642) has been found in locus DYS393, The highest gene diversity (0.825) has been found in locus DYS19, the reminds gene diversity (0.701, 0.730, 0.775) return to DYS390, DYS391, DYS392 respectively.

Figure (2) show distribution of allele frequency of each Y STR loci in Commoners sub-population, locus DYS390 has four alleles (23, 24, 25, 26), allele No. 24 has a highest frequency (0.389) and allele No. 23 has a lowest frequency (0.056). Locus DYS391 has four alleles (9, 10, 11, 12,), allele No. 11 has a highest frequency (0.347) and allele No. 9 has a lowest frequency (0.111). Locus DYS392 record five alleles (11, 12, 13, 14, 15), allele No. 11 has a highest frequency (0.333) and allele No. 14 and 15 have a lowest frequency (0.111). Other Y STR locus DYS393 has four number of alleles (11, 12, 13, 14) allele No. 13 has a highest frequency (0.486) and allele No. 11 has a lowest frequency (0.028). Finally, locus DYS19 has six number of alleles (11, 12, 13, 14, 15, 16) alleles No. 14 and15 have a highest frequency (0.222) and allele No. 16 has a lowest frequency (0.069).

The Gene diversity in Iraqi middle Euphrates sub-populations has been compared to the other areas population table (3) (Filiz *et al*., 2013; Jasem, 2013; Imad *et al*., 2014; share, 2014; Serkan *et al*., 2017; Tareq , 2017) showed that Iraq Arab male population share most of its predominant GD. The highest genetic diversity in all loci was in Middle Euphrates all population and Commoners sub-population, while Hashemites sub-population share some range of GD with other areas population, GD of locus DYS390 was near to Sudia arbia, Diyala, Baghdad, and Iraq population. In the locus DYS391 GD near to Al-Anbar population. GD of locus DYS392 was predominant on other populations. GD of locus DYS393 was near to Iraq Yazidi, Sudia arbia, UAE and Kuwait. While locus DYS393 GD was near to Iraq Yazidi.

In another study on 17 Y-STR Y-chromosomal short tandem repeat loci from the Cukurova region of Turkey the DYS391 recorded lowest gene diversity in this region was 0.51 and the highest 0.95 for DYS385a/b and no significant differences were found when compared this data with haplotype data of other Turkish populations (Serin *et al.,* 2011).

Y-STR Haplotype frequency, Haplotype Diversity and Discrimination Capacity

In the Hashemites sub-population goup of study (Table 4), the number of haplotype was 27, while the unique haplotypes were 19 haplotypes form (70.37%) and replicated haplotype were 8 haplotypes form (29.62%) from all population. The highest haplotype was (H4) which replicated six times and has frequency (0.083), as well as a haplotype (H11) was replicate five times and has frequency (0.069). While the haplotypes (H1, H5, H6, H16 and H18) were replicate four times (frequency of haplotype 0.056), the haplotypes (H25) was replicate three times and have frequency (0.042), finally nineteen haplotypes have frequency (0.014). Haplotype Diversity was (0.974). And Discrimination Capacity was (0.704) (Table 4).

In the commoners sub-population group of study (Table 4), the number of haplotype was 35, while the unique haplotypes were 33 haplotypes form (94.29%) and replicated haplotype were 2 haplotypes form (5.71%) from all population. The highest haplotype was (H42 and H57) which replicated three times and has frequency (0.042), while thirty-three haplotypes have frequency (0.014).). Haplotype Diversity was (0.999). And Discrimination Capacity was (0.943) (Table 5).

Y-STR Comparison of the haplotype diversity and Discrimination Capacity in different human population groups

The results of DC and HD of the present study were compared with other results from other populations (Table 6) UAE (*n*=436) (Zeyad *et al.,* 2020), Austria (*n*=425) (Pickrahn *et al.,* 2016), China (*n*=742) (Li *et al.,* 2020), KSA (*n*=597) (Khubrani *et al.,* 2018), India (*n*=259) (Mohapatra *et al.,* 2019), Kuwait (*n*=126) (Triki-Fendri, 2010), Iran (*n*=209) (Sayyari *et al*., 2019), Spain (*n*=146) (Saiz *et al*., 2019) and Turkey (*n*=86) (Ozbas-Gerceker *et al*., 2013).

The word haplotype describes a genetic unit or combination of alleles at adjacent locations or loci on the chromosome that are inherited together from a single parent. In this study HD was (0.997). When comparing HD with other countries it was less than UAE, China, KSA and Iran which was (0.999), but equalized to the Spain population (0.997), and more than Austria, India and Turkey which was (0.996) and Kuwait (0.994).

The DC calculated as a percentage of unique haplotype (means that the number of haplotypes observed only once in the population) by dividing the number of unique haplotype over the total number of the haplotype. DC of this study was (0.839), it was near to Kuwait DC (0.861), and upper than Austria DC (0.669) and India DC (0.787). But less than UAE, China, KSA, Spain, Iran and Turkey DC (0.962, 0.959, 0.909, 0.957, 0.976 and 0.937) respectively.

Table 1: Allele frequency and gene diversity of Hashemites sub-population (n=72)

Allele	DYS390	DYS391	DYS392	DYS393	DYS19
9	-	0.250	-	-	-
10	-	0.278	0.056	-	-
11	-	0.167	0.333	0.028	-
12	-	0.222	0.347	0.458	0.333
13	-	0.083	0.111	0.431	0.250
14	-	-	0.125	0.083	0.153
15	-	-	0.028	-	0.056

16	-	-	-	-	0.056
17	-	-	-	-	0.153
23	0.111	-	-	-	-
24	0.375	-	-	-	-
25	0.389	-	-	-	-
26	0.125	-	-	-	-
sum freq	1.000	1.000	1.000	1.000	1.000
N	72	72	72	72	72
NA	4	5	6	4	6
G D	0.690	0.787	0.747	0.605	0.784

*N=number of samples; NA=number of alleles of each loci; G D=gene diversity.

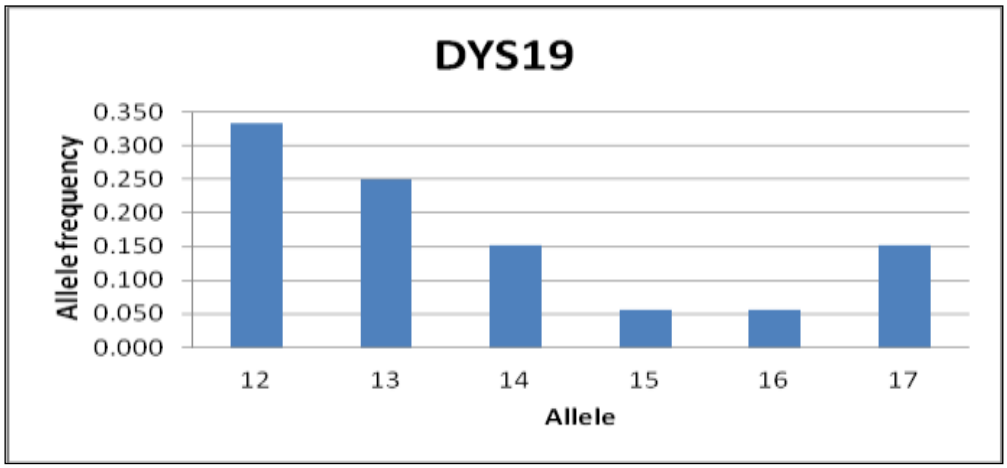

Figure 1: Allele frequency distribution of five Y STR loci. 4.5.3 Y-STR Allele frequency and Genetic Diversity for Commoners sub-population as a model DYS19

Table 2: Allele frequency and gene diversity of Commoners sub-population (n=72)

Allele	DYS390	DYS391	DYS392	DYS393	DYS19
9	-	0.111	-	-	-
10	-	0.278	-	-	-
11	-	0.347	0.194	0.028	0.181
12	-	0.264	0.333	0.319	0.111
13	-	-	0.250	0.486	0.194
14	-	-	0.111	0.167	0.222
15	-	-	0.111	-	0.222
16	-	-	-	-	0.069
23	0.056	-	-	-	-
24	0.389	-	-	-	-
25	0.292	-	-	-	-
26	0.264	-	-	-	-
sum freq	1.000	1.000	1.000	1.000	1.000
N	72	72	72	72	72
NA	4	4	5	4	6
G D	0.701	0.730	0.775	0.642	0.825

*N=number of samples; NA=number of alleles of each loci; GD=gene diversity.

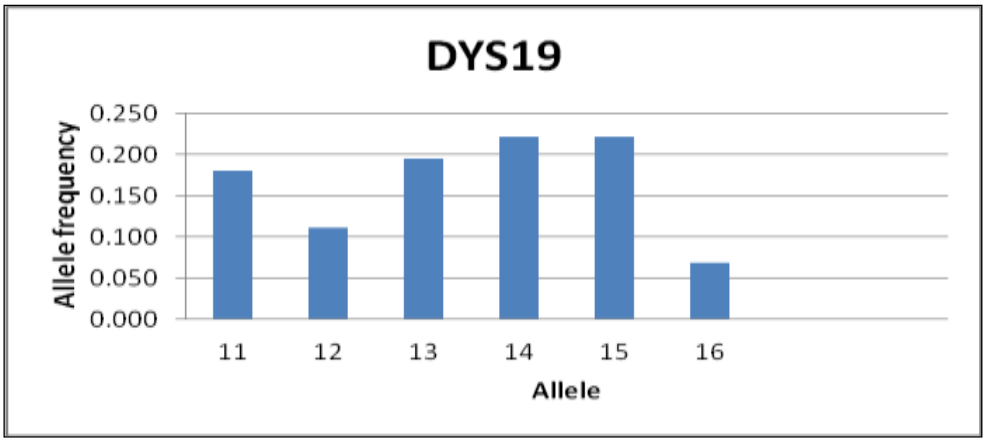

Figure 2: **Allele frequency distribution of five Y STR loci. As a model DYS19**

Table 3: **Comparison of Gene diversity with other areas population**

Area population \ Y STR loci	DYS390 GD	DYS391 GD	DYS392 GD	DYS393 GD	DYS19 GD
Middle Euphrates all population [1]	0.802	0.762	0.768	0.627	0.861
Hashemites sub-population [2]	0.632	0.618	0.788	0.676	0.765
Commoners sub-population [3]	0.803	0.773	0.750	0.613	0.844
South of Iraq[4]	0.598	0.534	0.373	0.575	0.572
Baghdad[5]	0.663	0.548	0.373	0.576	0.532
Al-Anbar[6]	0.572	0.606	0.230	0.519	0.472
Diyala[7]	0.647	0.588	0.253	0.639	0.603
Iraq[8]	0.657	0.580	0.269	0.514	0.665
Iraq Kurd[9]	0.708	0.493	0.514	0.585	0.634
Iraq Yazidi[10]	0.732	0.464	0.325	0.634	0.721
Saudi arbia[11]	0.663	0.493	0.354	0.651	0.600
Kuwait[12]	0.533	0.574	0.477	0.638	0.593
UAE[13]	0.707	0.455	0.402	0.641	0.643
Turkey[14]	0.700	0.550	0.450	0.580	0.640

1,2 and 3 (present study), 4,5,6 and 7 (Al-Zubaidi *et al*., 2019), 8 (Imad, 2014), 9 and 10 (Serkan *et al*., 2017), 11 (share, 2014), 12 (Jasem, 2013), 13 (Tareq, 2017) and 14 (Filiz *et al*., 2013).

Table 4: **Haplotypes and haplotypes frequency for the 5 Y-STR loci of Hashemites sub-population(H1-H10) and commoners sub-population (H28-H40).**

haplotype	DYS390	DYS392	DYS391	DYS393	DYS19	N	frequency
H1	23	12	11	12	12	4	0.056
H2	23	12	11	12	14	1	0.014
H3	23	11	9	12	15	1	0.014
H4	24	12	12	12	12	6	0.083
H5	24	11	12	13	12	4	0.056
H6	24	10	9	12	13	4	0.056
H7	24	13	10	13	13	1	0.014
H8	24	11	9	12	13	1	0.014
H9	24	11	12	13	13	1	0.014
H10	24	14	10	13	13	1	0.014
H28	23	11	9	12	13	1	0.014
H29	23	14	12	13	15	1	0.014
H30	24	13	9	13	12	1	0.014
H31	24	13	10	11	13	1	0.014
H32	24	12	11	13	13	1	0.014
H33	24	12	12	13	13	1	0.014
H34	24	15	9	14	13	1	0.014

H35	24	11	10	12	14	1	0.014
H36	24	11	11	13	14	1	0.014
H37	24	12	10	13	14	1	0.014
H38	24	12	12	13	14	1	0.014
H39	24	12	11	14	14	1	0.014
H40	24	12	10	12	15	1	0.014

Table 5: Comparison of the haplotype diversity and Discrimination Capacity in the study sub-populations groups

populations	All population	Hashemites sub-population	Commoners sub-population
Individuals number	144	72	72
Haplotypes number	62	27	35
Unique Haplotypes	52	19	33
Non-Unique Haplotypes	10	8	2
HD	0.997	0.974	0.999
DC	0.839	**0.704**	**0.943**

Table 6: Comparison of the haplotype diversity and Discrimination Capacity in different human population groups.

populations	Iraq[1]	UAE[2]	Austria[3]	China[4]	KSA[5]	India[6]	Spain[7]	Iran[8]	Kuwait[9]	Turkey[10]
Individuals number	144	436	425	742	597	259	146	209	126	86
Haplotypes number	62	419	329	700	541	188	139	209	101	79
Unique Haplotypes	52	403	220	671	492	148	133	204	87	74
Non-Unique Haplotypes	10	16	109	29	49	40	6	5	14	5
Haplotypes diversity HD	0.997	0.999	0.996	0.999	0.999	0.996	0.997	0.999	0.994	0.996
Discrimination Capacity DC	0.839	0.962	0.669	0.959	0.909	0.787	0.957	0.976	0.861	0.937

[1]Current study.; [2]Reference: (Zeyad *et al.,* 2020); [3]Reference: (Pickrahn *et al.,* 2016), [4]Reference: (Li *et al.,* 2020); [5]Reference: (Khubrani *et al.,* 2018); [6]Reference: (Mohapatra *et al.,* 2019); [7]Reference: (Saiz *et al*., 2019); [8]Reference: (Sayyari *et al*., 2019); [9]Reference: (Triki-Fendri, 2010); [9]Reference: (Ozbas-Gerceker *et al*., 2013).

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CONCLUSION

In this study the sub-population of commoners was more polymorphic in Y STR markers specially DYS19 and have high gene, Haplotype diversity and Discrimination Capacity than Hashemites sub-population.

CONFLICT OF INTEREST

None

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