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Study of mutant frequency hypoxanthine guanine phosphoribosyl transferase (HPRT) gene in human lymphocyte for local samples of Al-Tuwaitha region-Iraq

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Abstract: The aim of this study was determine the genetic damage in human blood lymphocytes for a number of 60 apparently healthy peoples were living Eshtar village and Al -Tameem region, near to the Iraqi Atomic Energy commission using Hypoxanthine guanine phosphoribosyl transferase (*HPRT*) mutation assay. Samples included 30 peoples from Eshtar village, aged (15-55 years) and 30 peoples from Al-Tameem region, aged (18 - 58 years), and 20 peoples aged (18 - 56 years) from other regions of Baghdad as a control group, was studied. The results of the average mutation frequency for *HPRT* (Mf-*HPRT*) gene showed a significant increase ($p<0.05$) in the males and females of human lymphocyte in this region as compared with the control group. In conclusion, the results indicated that there is a possibility of using the changes at the level of *HPRT* gene mutation as useful biomarkers for the detection of human exposure to radiological and chemical materials.

Key word: *Ionizing radiation, Human lymphocyte, HPRT mutation assay* .

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Introduction:

Hypoxanthine guanine phosphoribosyl transferase (HPRT) is a purine salvage enzyme that catalyzes the conversion of the purine bases hypoxanthine and guanine to the respective nucleotides inosine 5- monophosphate (IMP) and guanosine 5- monophosphate (GMP) (1). Human genetic studies indicated that the structural gene for HPRT is located on the X-chromosome (Xq27-28) (2). HPRT deficiency is the biochemical defect human Lesch-Nyhan syndrome (3), gout and hyperuricemia (4). Several studies of HPRT gene mutations in human cultured cells and lymphocytes *in vivo* have provided evidence for age, exposure and genetics to influence mutation frequency. An increased mutation frequency with increasing age in normal healthy people is generally observed (5,6). The approach for somatic mutation analysis in human has been widely used to determine *in vivo* background as well as acquired somatic cell mutation frequencies in pediatric and adult populations exposed to know and unknown environmental mutagenesis (7,8). Moreover, certain occupational exposures and life style factors such as smoking have been associated with an increased mutation frequency, while the intake of specific dietary items seems to have a protective effect against

mutations and cancer (9). The aims of the present study are to investigate the HPRT gene mutation in order to detect the effect of radiological and chemical hazard in human blood of Al-Twaitha region.

Materials and Methods:

Population Studies:

The study was conducted in 2015 by researcher examined 60 apparently healthy peoples previously living the Al-Twaitha region which surround to the Iraqi Atomic Energy Commission . These samples included 30 individuals selected randomly from population living in the Eshtar village (aged 15-55 years) and 30 individuals selected randomly from population living in Al-Tameem region (aged 18-58 years), compared with a sample of 20 healthy normal individuals collected randomly from population living in Baghdad for way for Al-Tuwaitha nuclear site (aged 16-56 years) as a control group . They were asked to fill in an extensive questionnaire which included the following criteria (age, sex, smoking drink alcohol, X-ray, abortion, time of living in these area, sort of work).

Blood Sampling:

Five ml of peripheral blood from all select subjects were collected and placed into sterile plain tube that contained lithium heparin. The blood was placed in a cool - box under aseptic conditions and transfer to the laboratory.

Blood Culturing:

The *HPRT* gene mutation assay was performed according to the description by Cao et al., 2002 (10) . Two sets of cultures were prepared, each set of culture contained 0.5 ml heparinized blood and 4.5 ml tissue culture media type RPMI 1640 with 20% fetal calf serum and 0.2 mg/ml phytohemagglutinine (PHA-M) .One set of culture was added with 0.2 mM 6-thioguanine (Sigma). At 33 h of incubation, cytochalasin B (the final concentration,4.5 mg/ml) was put into two sets of cultures. At 72 h of incubation,the lymphocytes were harvested by centrifugation and fixed with methanol: acetic acid (3:1). The slides were prepared and stained with 10% pH 6.8 Giemsa solutions.

Microscopic examination:

The binucleated and multinucleated cells per 1000 lymphocytes in two sets of cultures were scored under light microscopy (magnification 1000X). Mutant frequency of HPRT gene (Mf-HPRT) was calculated with the following formula (10):

Mf-HPRT(‰)=

$$\frac{\text{binucleated and multinucleated cells in culture with 6-TG per 1000}}{\text{binucleated and multinucleated cells in culture without 6-TG per 1000}} \times 1000‰$$

Data Analysis and Statistics:

The data of HPRT gene study were compiled into the computerized data file and frequency, distribution and statistical description (Mean, SE) were divided using SPSS statistical software. Using statistical analysis of variance (ANOVA) test and least significantly difference (LDS) test by probability of less than 0.05 ($p<0.05$) according to Duncan 1956 (11).

Results and Discussion:

The mutant frequencies of HPRT (Mf-HPRT) gene were performed on

peripheral blood lymphocytes which were obtained from 60 individuals of resident living the Al-Twaitha region such as Eshtar village and Al-Tameem region, then compared with 20 individuals control living in Baghdad a way from Al-Tuwaitha nuclear site . The *HPRT* gene mutation assay was performed according to the description by Cao et al., 2002(10). The Mf-HPRT gene was calculated binucleated, trinucleated and quadrinucleated lymphocyte cell per 1000 lymphocytes in tissue culture with and without 6-thioguanine.

The results of HPRT gene mutation assay in residents living these region surround to Al- Twaitha nuclear site are shown in table (1). The average Mf-*HPRT* (Mean ± SE) for Eshtar village and Al-Tameem region were 0.920 ± 0.0073 %, and 0.962 ± 0.0153 % , respectively when compared with the control (0.821±0.0089%). A significant increase (P< 0.05) in Mf-*HPRT* gene was observed in this region as compared with the control. However, no significant variation (P > 0.05) was observed in Mf-*HPRT* gene of Al-Tameem region, when compared with the Eshtar village Table (1). These results suggest that the *HPRT* mutation spectrum can be used as a potential biomarker for assessing a specific environmental risk. Furthermore, studies of mutations at *HPRT* gene have provided insights into several aspects of somatic mutations *in vivo*, including molecular mechanisms of mutagenesis, the relationship between DNA damage and mutation, as well as individual susceptibility factors such as DNA repair capacity (12).

Table (1): Mutant Frequency- *HPRT* gene (Mean ± SE) in the males and females of human lymphocyte resident living the Al- Tuwaitha region and control groups.

Region	Age (year)	No. of samples	Mutant Frequency-*HPRT* (Mean ± SE)
Eshtar Village	15-55	30	0.920±0.0073[a]
Al-Tameem region	18-58	30	0.962±0.0153[a]
Control (Baghdad)	16-56	20	0.821±0.0089[b]

♦Similar latter in a column (for comparison between regions) mean there is no significant difference (p < 0.05), according to Duncan test.

In this investigation, DNA damage, chromosomal damage and gene mutation of healthy normal individual's inhabitants living the Al-Tuwaitha region were detected with *HPRT* gene mutation assay. The results showed that the cytogenetic markers of individuals inhabited living in the Al-Tuwaitha region significantly increased as compared with the controls.

Table (2) shown that the Mf-*HPRT* gene was significantly higher (P< 0.05) in the male of resident living the Al-Tuwaitha region than the males of control, but the difference between Eshtar village and Al-Tameem region for Mf-*HPRT* gene were no significant (P > 0.05). The mutation at HPRT was examined in population residents living the Al- Tuwaitha region in this study , HPRT is the most commonly used genetic locus in mammalian cells mutational studies. The gene encoded for the purine salvage pathway enzyme HPRT. Hence, this locus is reasonably important to be tested because it is X-

linked and mutation at the HPRT (13). This result is similar to those of (14) in which the evaluation of HPRT mutant assay as a biomarker monitoring the specific environmental mutagen.

Table (2): Mutant Frequency- HPRT gene (Mean ± SE) in the males of human lymphocyte resident living the Al- Twaitha region and control.

Region	Age (year)	No. of samples	Mutant. Frequency-*HPRT* (Mean ± SE)
Eshtar Village	15-50	18	0.928±0.0106 [a]
Al-Tameem region	18-55	16	0.949±0.0168 [a]
Control (Baghdad)	16 -52	10	0.841 ± 0.0120 [b]

♦Similar latter in a column (for comparison between regions) mean there is no significant difference ($p < 0.05$) , according to Duncan test .

Table (3) shown that the Mf-*HPRT* gene was significant increase ($P< 0.05$) in the females of resident living in this region when compared with females of control. Also, the Mf-*HPRT* gene showed different significant ($P < 0.05$) in female living in the Al-Tameem region, as compared with the female residents living in Eshtar village Table(3). In the present study, the Mf-HPRT gene increase in the population of the Al- Twaitha region, due to the presence of low doses radioactive materials in this region because of its proximity to the nuclear site in that region .In the study of detection of radiological and chemical of public exposure to uranium-235 at Al-Tuwaitha nuclear research site (15,16). Moreover, certain occupational exposures and life style factors such as smoking have been associated with an increased mutation frequency (5, 6), while the intake of specific dietary items seems have a protective effect against mutations and cancer (9).

Table (3): Mutant Frequency- HPRT gene (Mean ± SE) in the females of human lymphocyte resident living the Al- Tuwaitha region and control.

Region	Age (year)	No. of samples	Mutant Frequency- *HPRT* (Mean ± SE)
Eshtar Village	17-45	12	0.0130 [a] ± 0.905
Al-Tameem region	25-58	14	0.981 ± 0.0239 [b]
Control (Baghdad)	17 -56	10	0.820±0.0145 [c]

♦Similar latter in a column (for comparison between regions) mean there is no significant difference ($p < 0.05$) , according to Duncan test .

Table (4) shown that the Mf-*HPRT* gene was significant increase ($P < 0.05$) in the males of residents living in Eshtar village as compared with females of this region. Also, the Mf-*HPRT* gene showed no significant difference ($P > 0.05$) between male and female in the Al-Tameem region and control. The difference in mutation frequency may be due to a different sensitivity to environmental mutagen for males and females. This result of the current study has been compatible with other study that low doses of ionizing radiation increase *HPRT* mutant frequencies of lymphocytes (17,18). In conclusion, the

results of our experiment suggest that the accumulation of genetic damage is detectable in peripheral lymphocytes of Local Samples of Al-Tuwaitha region. As well as , The results indicated is a possibility that there of using the changes in the level of *HPRT* gene mutation as biomarkers for the assessment of DNA damage in the human peripheral blood lymphocytes of population living in the Al- Twaitha region.

Table (4): Comparison between gender and mutant frequency- *HPRT* gene (Mean ± SE) in males and females of human lymphocyte resident living the Al- Tuwaitha region and control.

Region	Age (year)	No. of samples	Gender	Mutant Frequency-*HPRT* (Mean ± SE)
Eshtar Village	17-45	12	♀	0.0130 ± 0.905
	15-50	18	♂	*****0.928±0.0106
Al-Tameem region	25-58	14	♀	0..981 ± 0.0239
	55-18	16	♂	0.949±0.0168
Control (Baghdad)	175-8	10	♀	0.841 ± 0.0120
	16 -52	20	♂	0.820±0.0145

***** Significant differences between genders in this region and control using F-test.

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