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RESEARCH ARTICLE

RELATIONSHIP BETWEEN *H-RAS* ONCOGENE AND UTERINE CANCER

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ABSTRACT

The current study is skillful to detect of the mutations of the *H-RAS* oncogene in patient of uterine cancer. Thirty specimens of blood and tissue were collected for DNA extraction, H-RAS oncogene amplification and histopathology examination. The results revealed to the present of mutations in *H-RAS* oncogene represented by homozygous wild type Leu / Leu was 20%, mutant translocation genotype Val / Val was 48%, mutant deletion genotype Val / Leu was 22% and heterozygous genotype Leu / Val was 10%. The histopathological results of this study reveal to many types of uterine cancer. The major types of uterine cancer that appear in this study represented by endometrial adenosquamous carcinoma that associated with endometrial lieomyosarcoma, the other cases were represented as endometrial spheriodal cell carcinoma and as endometrial intra epithelial carcinoma.

INTRODUCTION

Uterine cancer begins when normal cells in the uterus change and grow uncontrollably, forming a mass called a tumor. There are two major types of uterine cancer, Adenocarcinoma, and this type of cancer makes up more than 95% of uterine cancers; It develops from cells in the lining of the endometrium. This cancer is also commonly called endometrial cancer. Sarcoma is the second form of uterine cancer develops in the myometrium or in the supporting tissues of the uterine glands (Cancer_Net .mht, 2011). It has been reported that the ras gene family (*K-RAS, H-RAS,* and *N-RAS*) is associated with the development of human neoplasms (Barbacid, 1987) Single-point mutations of the ras gene, usually at codons 12, 13, and 61, result in a single amino acid substitution in critical domains, and this substitution has a significant role in tumor development by making the proteins no longer dependent on GTPase (guanosine5'-triphosphatase)-activating protein regulation. Frequent ras mutations have been reported in a number of human cancers, including adenoca- rcinoma of the pancreas (90%), colon (50%), thyroid (50%), and lung (30%) (Bos, 1989). The *H-RAS* gene commonly is activated in human urinary tract tumors. H-RAS mutations have been reported in malignant fibrous histiocytoma (MFH), leiomyosarcoma, and rhabdomyosarcoma as for sarcomas (Wilke *et al.,* 1993; Bohle *et al*. 1996; Yoo and Robinson, 1999; Yoo *et al*, 1999).

MATERIALS AND METHOD

A total of thirty patients women with uterine cancer were contacted after surgery. The blood samples were collected in sterilized tube with EDTA, brought to the laboratory and kept directly in -20^{o}C till used for DNA extraction, while the tissue samples were collected from endometrial cancer for histopathological study and kept in formalin 10% for 48 hour(Luna,1968). Genomic DNA was isolated by DNA extraction kit. The mutation of the codon 12 of *H-RAS* oncogene was studied according to protocol of Chikako *et al.,* (1994).Briefly genomic DNA was amplified using the primers Forward5´- CTCTATAGTGGG ATCATAC-3´,Reverse5´-GACTCCTACCGGA AAC AGG-3´. PCR reaction mix and condition are PCR green master mix12.5 µl, Primer forward1 µl, Primer reverse1 µl, DNA5 µl, D.W. 5.5µl and 25 µl mineral oil. PCR conditions were denaturation 94ºC for 5 min 1 cycle, denaturation 94 ºC for 1 min annealing 58ºC for 1.5 min, extension 72ºC for 2.5 min. and 30 cycles and extension 72ºC for 5 min 1 cycle. The PCR product was 108 pb, then subjected to electrophoresis on a 2% agarose gel. PCR product was digested for 3 h at 37ºC with *Eco R1* restriction enzyme using 3µl of NE buffer 2, 0.5µl (5 units) of enzyme and 10µl of PCR product, 0.3µl BSA. The PCR products were classified as homozygous wild type (80-28 bp), translocation mutant genotype (108 bp), heterozygous (108-80 bp) alleles, deletion mutant genotype (80 bp).

RESULTS AND DISCUSSION

The results showed that the mutations of the *H-RAS* oncogene as presentation in the fig.1. The frequency of patient with uterine cancer *H-RAS* oncogene homozygous wild type Leu/Leu was 20% while that of mutant translocation genotype Val / Val was 48%. The mutant deletion genotype Val / Leu was 22%. The heterozygous genotype Leu / Val was 10%. The histopathological results of the current study revealed to many types of uterine cancer (Table 1). Twenty six cases from the total were represented by endometrial adenosquamous carcinoma that associated with 16 cases endometrial

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lieomyosarcoma(Fig.2,3,5,6 & 9) three cases were represented as endometrial spheriodal cell carcinoma(fiG.4&7). One case was appear as endometrial intra epithelial carcinoma (Fig.8)

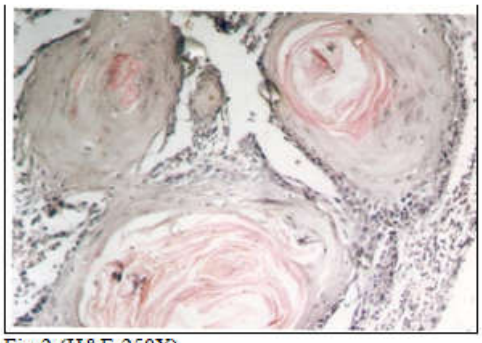
Fig.2 (H&E 250X)

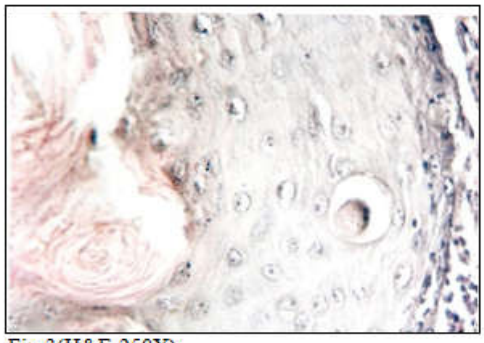
Fig.3(H&E 250X)

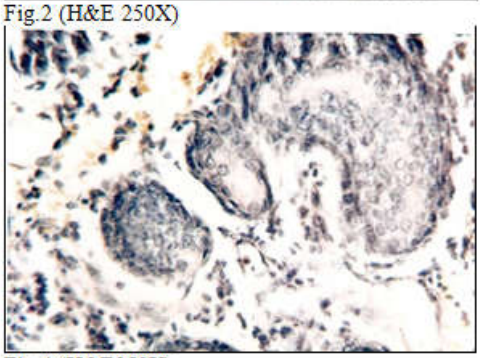
Fig.4 (H&E250X)

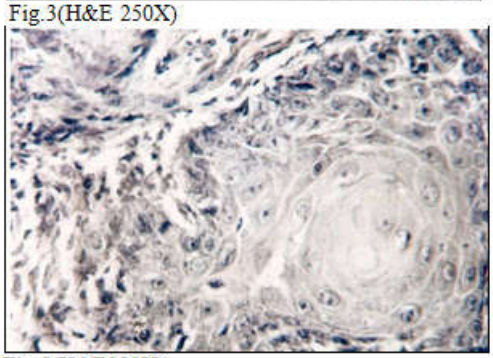
Fig.5(H&E280X)

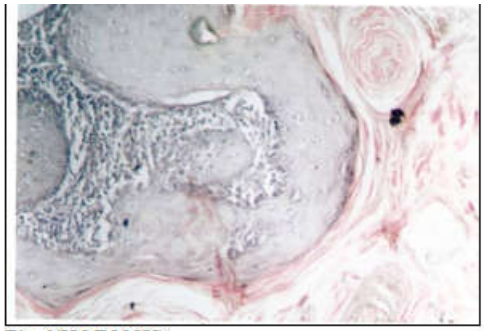
Fig.6(H&E200X)

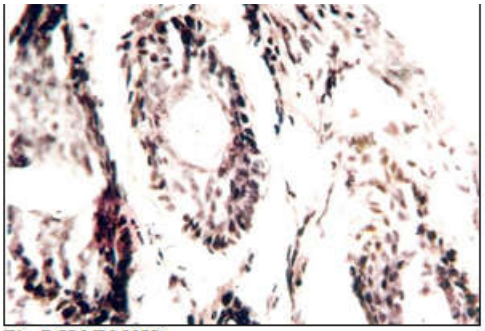
Fig.7(H&E250X)

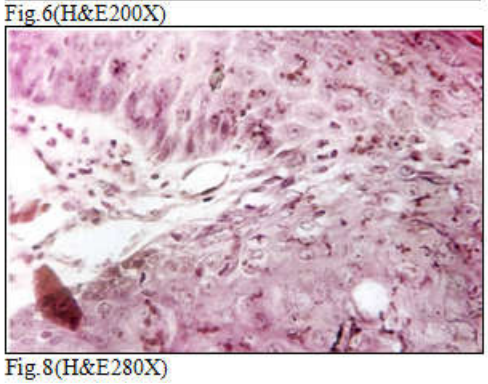
Fig.8(H&E280X)

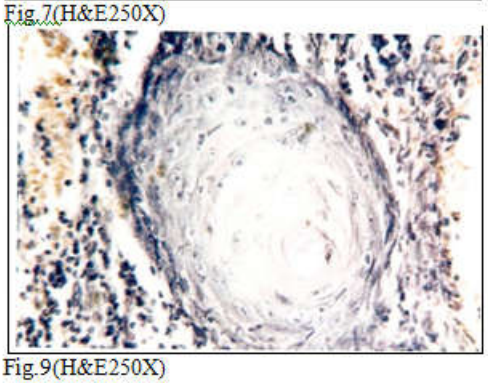
Fig.9(H&E250X)

GTPase *H-RAS* is involved in regulating cell division in response to growth factor stimulation. Growth factors act by binding cell surface receptors that span the cell's plasma membrane. Once activated, receptor stimulate signal transduction events in the cytoplasm, a process by which proteins and second messengers relay signals from outside the cell to the cell nucleus and instructs the cell to grow or divide. The *H-RAS* protein is a GTPase and is an early player in many signal transduction pathways and is usually associated with cell membranes due to the presence of an isoprenyl group on its C-terminus. Somatic mutations in the *H-RAS* gene are probably involved in the development of several other types of cancer. These mutations lead to an *H-RAS* protein that is always active and can direct cells to grow and divide without control. Recent studies suggest that *H-RAS* mutations may be common in thyroid and kidney cancers. The *H-RAS* protein also may be produced at higher levels in other types of cancer cells (Wong *et al.,* 1981; Russell *et al,* 1996).

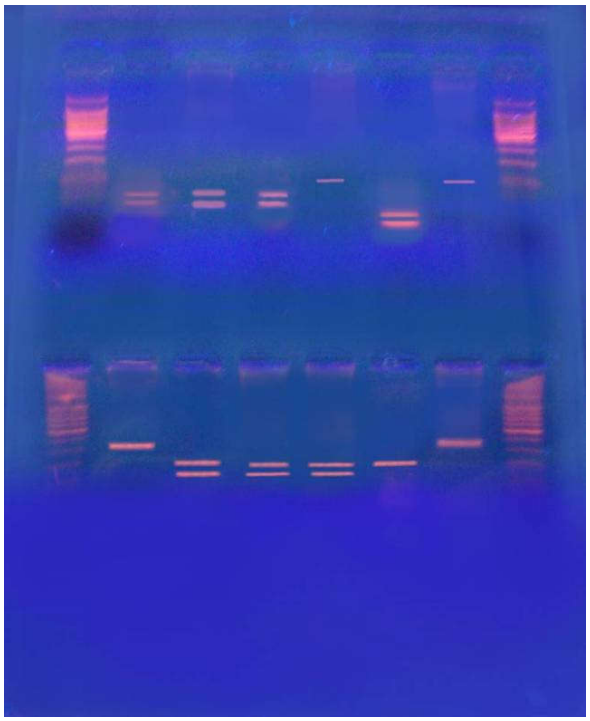

Fig. (1): PCR analysis of the codon 12 of *H-RAS* oncogene using *Eco R1* restriction enzyme. lane 1, 8,9,16 ladder, lane14 mutant deletion genotype, lane5,7,10&15 mutant translocation genotype, lane 6,11,12&13 homozygous wild type , lane 2,3&4 heterozygous genotype.

Table 1. The type and percentage of uterine cancer

Type of uterine cancer	Number of cases 30	Percentage%
Endometrial adenosquamous carcinoma with Endometrial lieomyosarcoma(16)	26	86.667
Endometrial spheriodal cell carcinoma	3	10
Endometrial intra epithelial carcinoma	1	3.333

*16 cases of endometrial lieomyosarcoma associated with the 26 cases of endometrial adenosquamous carcinoma

Since *RAS* communicates signals from outside the cell to the nucleus, mutations in *RAS* genes can permanently activate it and cause inappropriate transmission inside the cell, even in the absence of extracellular signals. Because these signals result in cell growth and division, dysregulated *RAS* signaling can ultimately lead to oncogenesis and cancer (Goodsell, 1999). Activating mutations in *RAS* are found in 20-25% of all human tumors and up to 90% in specific tumor types(Downward, 2003) Cells become cancer cells because of damage to DNA. DNA is in every cell and directs all its actions. In a normal cell, when DNA gets damaged the cell either repairs the damage or the cell dies. In cancer cells, the damaged DNA is not repaired, but the cell does not die. Instead, this cell goes on making new cells that the body does not need. These new cells will all have the same damaged DNA as the first cell does. People can inherit damaged DNA, but most DNA damage is caused by mistakes that happen while the normal cell is reproducing or by something in our environment Sometimes the cause of the DNA damage is something obvious, like cigarette smoking; but often no clear cause is found(American Cancer Society, 2011). The present study showed that there was a significant increase in accidences of uterine cancer in Iraq women may be due to the exposure to war weapons pollutant during war. Also the bioaccumulation of pollutants from the environment to the water and diet such oil ship leak, sewages drains, pesticides , herbicides and use the poisonous chemicals may increase the incidence of endometrial cancer(Al-Kurishy, 2008).

Conclusions and recommendations

Increased the risk of utrine cancer incidence in women who had mutant translocation genotype deletion and heterozygous genotype of *H-RAS* oncogene. As most of the cases were endometrial adenocarcinoma with lieomyosarcoma. This study recommended that women should be decreased exposed to radiation, chemical hazardous substances, treatment with hormones which enhancement the mutation.Performance another study to know the relationship between endometrial cancer and another related genes.

Acknowledgment

After praising God, I wish to express my deep gratitude to Dr.Khitam Jassim Salih whose to supply me with the patient uterine cancer tissues and blood specimens which are a part of specimens that used during her PhD study.

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