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Association of MET12 Gene Mutation with the Benign Breast Cancer in Iraqi Woman

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Abstract

The most prevalent form of benign breast cancer is Fibroadenoma, in fibroepithelial tumorigenes is the gene which play a key function is asomatic mutation in the MED12 gene (mediator complex subunit 12). The present research examined thebenign tumor tissue from I raqi women's for existence of the MED12 gene mutations or not. Total samples tested by reaction-Sanger chain polymerase sequencing was 100 (50 fibroadenoma and 50 healty blood as a control).Results showed that the variant represent35% (7/20) of fibroadenoma samples. consist of six unique intronic mutations And 14 unique exonic differences, These results suggest that this gene cannot consider as aindicator for the pathogenesis of fibroadenoma and do not have a role in its incidence.

Key words
Benign breast cancer, fibroadenoma (FA), Sanger sequencing, genetic modification, fibroepithelial lesions.

Introductions

The death of women suffering from cancer may arise from breast cancer[1&2]. However, the recognizing the benign breast tumors from malignant one is important point[3]**.**"the trumaof the breast fibroepithelialcan arise from avaried group of cancers having two phase with stromal and epithelial, referring to a broadgroup of biological mannerand changes in clinical management [4].

Fibroadenomas are the most public benign pathology of the mammary gland, it represent 50% of all biopsies of benign breast cancerand about 75% raisedlevel of biopsies in women under 20 years [5].The Mediator complex adjust the transcription by linking between RNA polymerase II initiation complex and DNA regulatory elements [6], work as both transcriptional activator and repressor,depending on the factors with which it interacts [7].

"MED12 gene is situated on Xq13.1 and comprises 45 exons. The recurrent of MED12 mutations and genetic changes in FAs was identified recently, which it predictable to be a seriouscause of genetic modifications in the occurance of FA Depending on physical nature and high occurrence, [8].Two different forms of X-linked main mental retardation, LujanFryns and Opitz-Kaveggia syndromes resulting fromMutations in MED12 [9]. Previous studies propose that leiomyoma-linked mutations in MED12 are involved in the stimulation

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of Wnt pathway leading to an impaired regulation of cell growth and tumor genesis [10 &11]. Decrease in CDK activity lead to somatic MED12 gene mutations resulting in impaired interaction between MED12 and Cyclin C-CDK8/19 [12].Also, several studies have proposedMED12 genes associatedwith benign fibrotic diseases [13]. The current study designed to evaluate the role of mutation in MED12 gene with occurrence of benign breast cancer inIraq women".

Materials and method

Specimens collection

Specimens were collected from kindi Hospital, Baghdad, Iraq. Fresh tissue specimens (50 benign) and (50 bloodsample as a controls) were taken from the women.The collected Tissue (1–10mg approximately) was placed in vials filled with normal saline and stored at −70◦C until they used for further analysis.

DNA extraction

The organic phenol- chlorophorm method was used for DNA extraction from tissue and blood specimens according to [14]with modification were accomplished,the addition of 10ml(10mg/ml) from proteinase K to the tube containing the sample withaddition stain buffer (0.5ml), instead of 1hr incubation after mixingincubate overnight at 56 c, thenthrown out the substrate and addition (0.5ml) of phenol-chloroform, vortex until emulsion was formedfor 15 sec. cooled absolute ethanol (1ml)was added to tube andcentrifuged for 12min at 15000 rpm, before DNAwas eluted in 50 µl of TE, 1ml of 70% ethanol was added and the tube centrifuged at 10000 for 5 min. finally the NanoDrop (Thermo Scientific, USA) was used for quantification of both concentration and purity of DNA, while the integrity was examined after electrophoresis the extracted DNA on 0.8% agarose gel electrophoresis.

PCR amplification

Polymerase chain reaction was performedin an applied biosystem thermal cyclerby using the primer pair stated by the [8] for studying the genotyping the MET12 mutation, the total reaction volume was 25 µl containing 0.5 µl of each primer (Canada),the forward 5′- AACGTAAGGGCCCAGCTTTA- 3′and the reverse one is 5′- CAGGGCCTTTGCTCCTTCTTA- 3′, and 12.5 µl of master mix (Promega, USA), and 3 µl of DNA sample. The PCR conditionincluding an initial denaturation at 94°C for 5minutes , followed by30 cycles of94°C denaturation for 30seconds, annealing at 60°C for 30seconds, and final extension at 72°C for 45seconds. By using the 2 % agarose gel electrophoresis,the amplified products were analyzed. Gel documentation system was used for imaging the gel and to determine the amplicon lengths.

Genotyping by sequencing

To determine if the mutant alleles ofMED12 are expressed in the benign tumor cell or not, forty samples were sent for sequencing, twenty from patients group and twenty from control group. The amplified fragments were sequenced on AB13730XL Applied Biosystems machine in NICM/USA Company. depending on BLAST (basic local alignment search tool)

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program,A homology search was achieved that is available online at (http://www.ncbi.nlm.nih.gov)at the National Center Biotechnology Information (NCBI) and BioEdit program. The obtained results were matched with data obtained from Gene Bank published graphic program at the NCBI online.

Results and Discussion

DNA extraction and PCR amplification

The fresh tissue samples collected from the patients and blood sample belong to the control subjects was usedas a source to obtain a pure DNA for PCR amplification. The results revealed that enough DNA concentration for PCR amplification was produced from both the fresh tissue and blood samples (Fig 1), it was in 150-350 ng / µl ranged with an 1.8 – 2purity range.

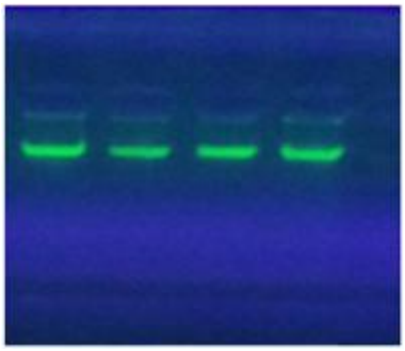

Figure 1> Gel electrophoresis of genomic DNA extracted from tissue sample on 0.8% agarose gel at 5 v/cm for 1hrs, visualized under UV after staining with red safe.

Using the primers pair mentioned in the previous study [8] for PCR amplification the results revealed the presence of clear band with 322bp in size as illustrated in fig. 2.

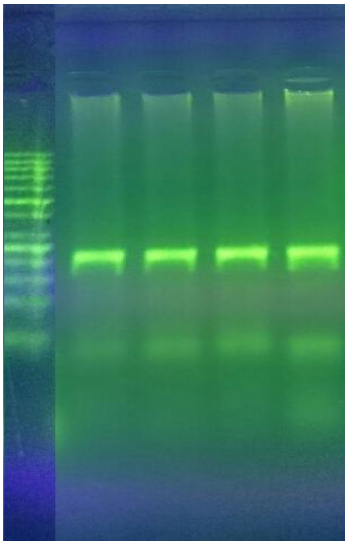

Figure 2. gel electrophoresis of PCR product (322bp) for MED12 gene, on (2%) agarose gel and run at 100 volt/ cm for 2 hours. DNA marker (50bp).

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Genotype analysis of MED12 gene

By using AB13730XL applied biosystems machine the genotyping analysis fortheMED12 gene was done and depending on basic local alignment tool (BLAST) program available at the national center biotechnology information (NCBI) and BioEdit programs, the Homology search was achieved. The obtained results were compared with the obtained data from the gene bank of the apparently healthy control which is available online at the NCBI under the reference sequence ID: NG_012808.1, the genetic variationsin the control samples stated that there was no mutation was recorded while in the FA samples in the exon 2variation was established, Overall, 35% (7/20) genetic variation as illustrated in (Table 1).The results referred that the incidence of benign breast cancer in Iraqi patients not related with MED12 gene mutation.

Table 1: genetic variation for MED12 gene from cancer patients

Variation	Number of patients
c.165G>T, and c.205G>T; and two deletions c.124_159 del	4
five SNPs c.122 T > C, c.126 A>T, c.130G>A	2
c.171delC, and six unique intronic mutations include two SNP c.33 +477 T>G and 33+429 A>C; one insertion c.33+476_477 G ins	1

The obtained percentage was near to that published by the researcher[15], they evaluated the mutations of MED12 gene in uterine leiomyomas of Iranian women, they stated that there are eleven positive mutation lesion, about 7 of them reported in codon 44 in a heterozygous manner and missense mutation type aremore common, also, they revealed that the most prevalence one is c.131G> A. and after they displayed that 47.8% of Iranian patients havenmutation positive lesions, approving the variety between the populations and depending on other researchers which related the incidence of MED12 mutations to the ethnic differences, as the obtained results in this study shows fewer mutation positive lesion than published data confirming the diversity between the populations.

The results mentioned in this study, proposed that incidence of breast fibroadenoma may be related to themutation in MED12 gene, also several studies like (11&16) stated that mutation in exone 2 was involved in the pathogenesis of uterine fibroids, as well as in a study on Japanese women establish this association (17). This association can be interrupted by the fact that this gene play as an important factor in many cellular signaling mechanisms that interacting with multiple receptors such as estrogen receptor (18). Researchers in (5 & 19)mentioned that in depending on the pathology the expression of MED12 was differ, at the same time the mutation was vary according to racial origin, this can interrupted the difference in mutation reported in our study in comparison with that of other population

Mediator is huge macromolecular complex with versatile functions having at least 31 subunits. The latter section contains MED12, MED13, CDK8, and cyclin C [20]. The mediator complex is a essential coactivator of transcription. The precise mechanisms by which mediator regulates Pol II activity remain unwell understood, but it is well recognized that mediator, with its kinase activity, can regulate phosphorylation of the C terminal domain of RNA polymerase II. Therefore, any alteration in MED12 disrupting the kinase module can have adverse effects on its regulatory functions. Both exons 1 and 2 encode the cyclin C binding domain of MED12. Therefore, mutations in these exons disturb MED12 cyclin C binding and result in reduced affinity for cyclin C-CDK8 and loss of mediator-associated CDK function [21&22]. So, the proper exon sequence is important for the protein's function[23].

Occurance of *MED12* mutations have also been recognized in leiomyomas of the uterus, with reported frequencies of 52–82% in addition to fibroadenomas, [13; 24;19]. The mutation spectrum in in fibro epithelial tumours of the breast is similar to that observed uterine leiomyomas. Such asThe comparable mutation patterns among these tumours indicate a common biological role of *MED12* mutation in the tumourigenesis of these tumours [25].

Conclusion

It can be concluded that the MED12 gene mutation was not associated with occurrence of fibroadenoma breast cancer in Iraqi population

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