Sequencing and structure analysis of UBC gene for breast and lung cancer

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<th>Article History</th>
<th>ABSTRACT</th>
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| Received on: 15.03.2019  
Revised on: 22.06.2019  
Accepted on: 26.06.2019 | In the present study, sequencing approach has been adopted for exploring the genetic alteration of sequences for the ubiquitin gene (UBC) in patients of breast and lung cancer and comparing the results with a normal sequence that obtained from NCBI. The aim of this study was to detect for genetic alterations of UBC gene in the breast and lung cancer patients then compare with healthy control subjects, to investigate the association between the mutations at the intron region of the UBC gene and cancer disease, 40 blood samples were examined from patients with breast and lung cancer aged ranged from (17-65) years, were collected at Al-Amal Hospital of cancer in Baghdad province/Iraq, the period of collecting samples were from October/2018 to January/2019. While twenty-two blood samples from healthy control subjects were collected at ages ranged from (19-59). After DNA extraction, the PCR primer was designed to amplify the region in the UBC gene (part of exon 1 and the whole intron). Here we report the polymorphism of the intron sequence of the UBC gene in Iraqi population as the results of sequencing the PCR amplified products showed three different transition mutation G→A, C→T, T→C in patients with breast cancer were also appeared in healthy control subjects. While nine transition mutations appeared in lung cancer patients, at different locations of the sequence were detected by BLAST tool. |

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ISSN: 0975-7538  
DOI: https://doi.org/10.26452/ijrps.v10i3.1329

INTRODUCTION

Molecular genetic analysis help in early detection of diseases like cancers, if these were diagnosed at early stage and also can give the proper therapeutic for curing (Young et al., 2018). DNA mutations in blood circulation can be successfully detected by using the appropriate bio informatics techniques, hence it helps to find cancers early and give a chance for a variety of treatment (Heitzer et al., 2017).

The accumulation of misfolded or damaged proteins that are resulting from stress conditions increase the amount of insoluble proteins and play a role in cytotoxicity, change the individual sequence, contribute in causing of many diseases including cancer and induce cell death. Therefore, the need of an increase of ubiquitin level during stress conditions is necessary to produce poly-ubiquitin protein to degrade misfolded and damaged proteins (Kedves et al., 2017). Ubiquitin-protein composed of 76 amino acids and plays an important role with proteasome for destruction and removing harmful, unwanted, misfolded proteins and for the proteins that have unnecessary highly increasing. Ubiquitin is also involved in protein modification, and regulation of signal pathways, in spite of not fully completed understanding on several ubiquitin modifications (Swatek and Komander, 2016).
In mammals, four genes that encoded for ubiquitin protein; in 1985, Wiborg and his colleagues were the first that characterized these four genes. The first two genes are UBA52 and UBA80 that fused to the ribosomal proteins, while the second two genes UBB and UBC are polyubiquitin genes which encode three and nine head-to-tail repeats of ubiquitin, respectively (Wiborg et al., 1985).

The polyubiquitin gene (UBC) composed of a unique structure coding for polyubiquitin c protein that containing about eight to nine tandem repeats of the ubiquitin coding units. However, the reason behind this untraditional gene structure is still not understood yet. UBC has distinguished features that make a significant effect during cellular functions, mainly its responsibility for stress regulation in mammals. It is important to maintain its level in cells because any other Ubiquitin genes cannot replace it and considered as a fundamental source for Ubiquitin during stress and cell reproduction (Bianchi et al., 2018). In 1992 Board, P.G. detected cytogenetic location for UBC gene on chromosome band 12q24.3 which is the long (q) arm of human chromosome 12 at position 24.3 (at region two, band four, subband three) (Board et al., 1992). In 1996, Nenoi et al. determined the intron sequence with the two exons of which the first exon is non-coding for protein thus, it is not found in the mature mRNA molecule. Thereby, the untranslated region (UTR) in this gene consists of the intron and the first exon while the second exon has coding sequence of protein (CDS) (Nenoi et al., 1996). Bianchi et al., 2009, discovered the importance of the unique intron of human UBC gene, which is essential in enhancing the gene expression and transcriptional regulation (Bianchi et al., 2009). Any changes, even at one single genomic aberration of a potent mutation prevent normal folding of protein can causes cancer (Mansour, 2018). Cancer cells would try to make changes in the UBC gene or its expression protein by modifying dysregulating in order to access signal for oncogenes that cause development and progression of cancer. The concentrations of proteins are altered in cancer lead to Aggregation and accumulation of proteins that cause toxic effects; this occurs because of changing in transcriptional and translational processes hence, cancer cells will increase the activity ubiquitin protein (Wu et al., 2014). Any Errors in gene expression (transcription and translation) would increase the production of protein with the possibility of the generation of abnormal proteins. The aim of the current work was to characterization partial of the (UBC) gene sequence (the first non-coding exon and the intron) region of each group of breast, lung cancer patients and healthy control subjects, compare them with the reference sequence of the gene from NCBI which may closely relevant to detect the mutations of this gene and check for the effects of these mutations for cancer disease.

**MATERIALS AND METHODS**

**Blood Sample collection**

All samples from patients and healthy control were collected under the approved protocol of the Iraqi ministry of health. The sample size of this research paper is 62 samples, 40 human patients with breast and lung cancer (20 samples for each type) and 22 samples for healthy control subjects. For each sample drawn two milliliters (2ml) of whole blood collected from the venous blood under aseptic conditions then immediately transferred into sterile EDTA tube for a genetic test. All samples of blood were stored frozen at (-20°C).

**DNA extraction from blood samples**

The human genomic DNA was extracted from whole blood of breast and lung cancer patients and healthy control subjects by using the protocol in the QuickDNA™ Blood MiniPrep Kit (Catalog Nos. D3073/ Zymo Research/USA).

**Estimation of DNA concentration and purity**

The human DNA concentration and purity of the samples that extracted from whole blood were estimated by using (the Quantus™ Fluorometer) Promega/USA, this was first done by Calibrating the Quantus™ Fluorometer for Use with the Quantifluor® Dye Systems. The purity of DNA was determined at wavelength 260nm - 280nm, which represents the ratio of the absorbance.

**Agarose Gel Electrophoresis**

After the extraction of genomic DNA from blood, agarose gel electrophoresis was adopted to determine DNA fragments to confirm the presence and integrity of the extracted DNA. After pouring the gel, the samples were carefully loaded into the wells of the gel. Then An Electric current of 75 volts has been exposed for 1 hour till the tincture has reached to the other side of the gel. Afterwards, the gel was tested by a source of the UV at 336 nm after putting the gel in a pool contained on 30μl Red safe Nucleic acid staining solution and 500 ml from distilled water, the results were photographed.

**Primer design for UBC gene**

Primer for UBC gene that used in this study was designed by using the National Center for Biotechnology Information (NCBI) Primer design tool from the website: http://www.ncbi.nlm.nih.gov/tools/prim...
er-blast/, the designed primer is shown in Table 1. Then the primer provided by Bioneer Company/Korea in a lyophilized form and using the primers in PCR.

**Table 1: The sequence of the specific primer of UBC gene that used to amplify part of the first exon and the whole intron region of the UBC gene in this study with GC content and Tm**

<table>
<thead>
<tr>
<th>Prime</th>
<th>Sequence</th>
<th>Tm (°C)</th>
<th>GC (%)</th>
<th>Product size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forward</td>
<td>5’-AGA CCG AAA ACC TCG ACC CC-3’</td>
<td>57.3</td>
<td>55</td>
<td>1053bp</td>
</tr>
<tr>
<td>Reverse</td>
<td>5’-TCC GCT AAA TTC TGG CCG TT-3’</td>
<td>57.1</td>
<td>50</td>
<td></td>
</tr>
</tbody>
</table>

**PCR**

Maxime PCR Pre Mix kit was used to amplify the DNA target sequence of UBC gene. According to the instruction of the manufacture of Maxime PCR PreMix Kit (i-Taq) from INTRON (Cat. No. 25025)

The mixture of the specific interaction for diagnosis gene consists of 5μl Taq PCR Pre Mix 10 picomols/μl (1μl) of Forward primer, 10 picomols/μl(1μl) of Reverse primer, 1.5μl of DNA, 16.5 μl of Distill water the final volume is 25μl. The temperature has changed through the work of (Gradient PCR) for all samples to select the optimal condition, and also changed the concentration for DNA template between (1.5-2μl) where is considered these two factors from important factors in primer annealing with complement (Al-Radeef et al., 2018).

Agarose gel was prepared (8 grams of agarose melted in 100 ml (TBE) Tris-Borate EDTA Buffer) then added Loading buffer KAPA Universal DNA Ladder (100pb) (cat. no. KK6302) to determine the molecular size of the band. Carefully loaded (5) μl of the PCR amplified products into the wells of the agarose gel and set to run voltage at 75Volt for 1 hour. The gel was stained with Red safe Nucleic acid staining solution so the bands can be seen obviously.

**Results of DNA Extraction**

DNA was successfully extracted from all samples. The mean concentration of total DNA 175.35 ng/μl in breast cancer patient samples and 163.95 ng/μl in lung cancer patients, while in healthy control subjects 196.2 ng/μl. The purity DNA samples mean 1.81 ng/μl in both breasts, lung patients groups. The mean of control subjects was 1.83 ng/μl.

**UBC gene amplification by PCR**

Polymerase chain reaction technique (PCR) was used to perform the amplification of UBC gene by using set of primers that amplify the gene. All samples (patients and healthy control) showed positive results for the gene presence after analyzing on agarose gel, as illustrated in Figure 1.

The amplified fragment was electrophoresed on 2% agarose gel stained with Red safe Nucleic acid staining solution at 75 volts. 1x TBE buffer for 1:30 hours. M: DNA ladder (100). Lane 1-10 represent healthy control samples. Lane 11-20 represent breast cancer patient’s samples. Lane 21-30 represent lung cancer patients’ samples.

**Detection of UBC gene alteration of by sequencing**

The current study utilized forward primer of breast, lung cancer patients and healthy control human by using direct sequencing. To identify and study the difference between the sequences of UBC gene in the groups of this study, 20 μl of 15 samples which gave clear bands (5 samples for each group of breast and lung cancer patients and 5 samples of healthy control) of the product of the PCR reaction with the specific primer of gene UBC gene that were sent to Macrogen company/Korea, for sequencing the first exon and intron region of the UBC gene for each samples by using AB DNA sequencing system. The results have been retrieved as nucleotides sequence (in FASTA format) two weeks later.

After obtaining the results compared to the results, with original sequencing of gene UBC in the Internet (http: NCBI Reference Sequence) by using BLAST server (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (Zhao et al., 2000), for detection the location of mutations in UBC gene for patients.

**RESULTS AND DISCUSSION**

Intensive analysis of human UBC gene variation help to improve in treating genetic diseases that related...
with genetic alterations of ubiquitin gene like mutations and variations.

Ubiquitin-protein is considered conservative in cells of eukaryotes and found to be important in regulation of many functions within cells, therefore, any changes from genetic errors at molecular level of the ubiquitin genes especially (UBC gene) to the high level of expression of the ubiquitin protein (mainly polyubiquitin proteins) all these changes will cause many diseases including cancer.

The comparison between the nucleotides sequence of amplified the primer of breast cancer patient and reference sequence are shown in Figures 2, 3 and 1, and for lung cancer patients shown in Figure 3, while the comparison between nucleotides sequence of amplified the primer of control sample and reference sequence are shown in Figure 4.

Figure 2: Alignment between the sequence of breast cancer patient and Homo sapiens ubiquitin C (UBC), RefSeqGene on chromosome 12, Sequence ID: NG_027722.2.

Figure 2 shows that the substitution is transition type and found on locations: 6071, 5520, 5421 in the UBC RefSeqGene, the nucleotide transition is G>A, C>T, T>C respectively.

Figure 3: Alignment between the sequence of lung cancer patients group and Homo sapiens ubiquitin C (UBC), RefSeqGene on chromosome 12, Sequence ID: NG_027722.2.

Figure 3 shows that the substitution is transition type and found on locations: 6071, 5520, 5421 in the UBC RefSeqGene, the nucleotide transition is: G>A, C>T, T>C respectively.

Genotype analysis of UBC gene for the PCR products of the primer indicated much of genetic alterations. It was found at intron region: point mutations of the transition mutation type was detected at three locations G→A, C→T, T→C for breast cancer patients and found the same at the healthy control subjects while for lung cancer patients found 9 transition mutations at different locations all mutations are listed in Table 2.

The Genetic alteration that occurs in the sequence of the intron region has an effect on the transcription and translation processes that cause alterations in mRNA and can be correlated with many diseases (Zhao et al., 2000) (Subramanian and Kumar, 2003) (Hsiao et al., 2016). At the translation process the untranslated regions UTRs (including introns and non-coding exon) are involved in regulating the activity of a protein, therefore; mutations occur in these regions may affect on the synthesized protein units function (Vaz-Drago et al., 2017).

In this study the same substitution mutations that appeared in all samples of breast cancer patients and healthy control subjects are considering as polymorphism for Iraqi population for the intron region of the UBC gene (Collins and Schwartz, 2002).

The results of substitution mutations in lung cancer patients could be have a relation to causing cancer disease, hence the role of ubiquitin in development and treatment of cancer disease thus ubiquitin can considered to be as lung cancer target (Tang et al., 2015). The mutations on UBC gene that were recorded by the current study could play a role in changing of physiochemical properties for protein and structure as shown in the result of Sequence analysis and structure analysis these results agreed with studies (Sinha and Nussinov, 2001) (Venselaar et al., 2010) of which demonstrated that protein structure is affected by mutations and can cause al-
Table 2: Results of sequencing for the primer for the intron region, show point mutations (transition type) for groups of breast and lung cancer patients

<table>
<thead>
<tr>
<th>Samples Groups</th>
<th>Type of substitution mutation</th>
<th>Nucleotide</th>
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<tbody>
<tr>
<td>Breast cancer patients</td>
<td>Transition</td>
<td>G &gt; A</td>
</tr>
<tr>
<td></td>
<td>Transition</td>
<td>C &gt; T</td>
</tr>
<tr>
<td></td>
<td>Transition</td>
<td>T &gt; C</td>
</tr>
<tr>
<td>Lung cancer patients</td>
<td>Transition</td>
<td>G &gt; A</td>
</tr>
<tr>
<td></td>
<td>Transition</td>
<td>C &gt; T</td>
</tr>
<tr>
<td></td>
<td>Transition</td>
<td>G &gt; A</td>
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<tr>
<td></td>
<td>Transition</td>
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<td>Transition</td>
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<td></td>
<td>Transition</td>
<td>C &gt; T</td>
</tr>
<tr>
<td></td>
<td>Transition</td>
<td>T &gt; C</td>
</tr>
<tr>
<td>Healthy control subjects</td>
<td>Transition</td>
<td>G &gt; A</td>
</tr>
<tr>
<td></td>
<td>Transition</td>
<td>C &gt; T</td>
</tr>
<tr>
<td></td>
<td>Transition</td>
<td>T &gt; C</td>
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<td>Transition</td>
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<td>Transition</td>
<td>C &gt; T</td>
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<tr>
<td></td>
<td>Transition</td>
<td>T &gt; C</td>
</tr>
</tbody>
</table>

Termination in the sequences and entire function of the protein in addition to its activity.

CONCLUSION

Further studies should be considering sequencing of the whole sequence of the UBC gene including the coding exon would help better understand the complete effects of mutations in this gene.

CONFLICTS OF INTERESTS

All authors have none to declare

Author contributions

All author contributed equally

ACKNOWLEDGMENTS

The authors are thankful to the management of Biotechnology Division, University of Technology for providing basic facilities for this research work.


