Demographic and genetic study for a sample of Iraqi smokers

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Abstract: Abstract: To examine the relationship between smoking and genetic and demographic aspects, using statistical analysis and genetic techniques. Subjects and methods: One hundred and fifty of apparently healthy Iraqi heavy smoker volunteers in comparison with fifty of apparently healthy non-smoker volunteers as a control group. Information for demographic study was taken from smokers and non smokers subjects according to a questionnaire that included, name, gender, age, consumption of pack number per day and duration of smoking, in the period from the beginnings of March 2014 to the end of June 2016. Through the molecular study, DNA was extracted by using the genomic isolation kit, then subjected to PCR analysis by using four sets of primers, then the PCR product were sequenced to detect the *TP53* mutations. Results: The results of the demographic study revealed that the highest number of smokers located in the age group (36-45) represented 38 (25.33%) of the total number with significant difference (P≤ 0.05). The males constituted 91(60.67%) more than females 59 (39.33%) with the high significant (P≤ 0.01). The distribution of smokers according to pack consumption number by smokers a day showed that the highest number 134 (89.33%) consumed more than one pack per day against 16 (10.67%) of one pack a day with a high significant (P≤ 0.01). Moreover the highest number 46 (30.67%) had been smoking for (16-20) year, while the lowest number 22 (14.67%) of smokers had been smoking for (5-10) years with a high significant (P≤ 0.01). The results of genetic study showed the presence of many variations in different locations in *TP53* gene such as G to C polymorphism which were found in exon 5 with the percentage of (47.3 %) among smokers in comparison with non smokers control (0.0%). On the other hand, it was observed that exon 6 had deletion in a high frequency among smoker individuals at a percentage of (19.3%) rather than in the non-smokers (0.0%); however, no genetic variations were shown in exons 7 and 8.
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1. Introduction

Cigarette smoking responsible for 30% of all cancer deaths in developed countries (WHO, 1997). Because tobacco smoke contains over 7000 different chemicals, 69 of them have been classified as "carcinogenic to humans". So, the works of many chemists and biologists over the past 50 years have showing the harmful effects of many tobacco components the (American Joint Committee on Cancer,2010 and American Cancer Society, 2013), a proven result is mutagenesis, that is the ability to induce mutations. Cancer arises when mutations accumulate within DNA because some cells act as outlaws (Detterbeck *et al*., 2015).

The *TP53* gene has been a common target of mutational studies because its protein in a tetrameric complex binds specific DNA sequences and appears to be a transcription factor that may regulate the expression of other genes in either a positive or negative manner (Real, 2007). The *TP53* plays a role in gene transcription, DNA repair, cell-cycle arrest cell cyclin function, genomic stability, chromosoma1 segregation, senescence, and apoptosis (Harris, 1996).

Inactivation of *TP53* is critical significance in carcinogenesis as it not only leads to enhance replication of cells, other than in exacting, to enhance replication of DNA damaged cells, some of which may inactivated tumor suppressor genes or activated oncogenes, or both (Wender *et al*., 2013).

About half of all cancers examined have a point mutation or deletion in the *TP53* gene and most of these mutations give increase to amino acid changes in evolutionarily conserved sites of the protein, suggesting that they are functionally important changes (Roos and Kaina, 2006 and Hollstein *et al*., 2013).

In this study *TP53*gene was chosen due to its importance and relationship with cancer of smokers, in the same time there is rare or no studies concerning this gene with smoking in Iraq. Therefore, the principal aim of the study was to investigate the joint effect of tobacco exposure and alteration *TP53*gene in Iraqi smokers and distribution of smokers according to the age, gender, consumption of pack number smoking a day and duration of smoking.

2. Subject Materials and methods

One hundreds and fifty of apparently healthy Iraqi volunteers of heavy smokers for at less one pack per day and a period of not less than 5 years, and fifty apparently healthy Iraqi volunteers of non smoker subjects as control were employed in the study, with age ranged between (14-67) year and from both genders. A questionnaire form was filled for each volunteers. The Questionnaire included information index about the smokers and non-smoker volunteers such as age and sex. It is also included inquiry about the smoking years number and the numbers of pack per day.

DNA extraction

Two ml of blood were taken from all subjects, collected in EDTA anticoagulant tubes and subjected to DNA extraction as mentioned by standard protocol according to Sambrook and Russell (2001), using genomic DNA purification kit (Bioneer/ Korea).

Gel Electrophoresis.

Agarose gel electrophoresis, was adopted, after DNA extraction, to confirm the presence and integrity of the extracted DNA according to Sambrook and Russel (2001).

Estimation of the DNA concentration and purity.

Concentration and purity of the DNA were carried out according to Sambrook and Russell (2001), by using Nanodrop (BioNeer /Korea).

Amplification of *TP53* Exons

The amplification of *TP53*gene exons by PCR was done by using specific primers pairs which supplied by Alpha DNA company/ Canada, depending on NCBI and according to Mateen and Irshad (2015). The primers sequences were showed in table (1).

Table 1: Sequences of primers used in this study.

Primer	Size bp	Forward
Ex 5-F	588	TGTAAAACGACGGCCAGTGCTACAACCAGGAGCCATTGTC
Ex 5-R		CAGGAAACAGCTATGACCCACCTACCTGGAGCTGGAGCTTA
Ex 6-F	248	TGTAAAACGACGGCCAGTAGGCTAAGCTATGATGTTCCTTAGATTAGG
Ex 6-R		CAGGAAACAGCTATGACCTCCTGGTTGTAGCTAACTAACTTCAGA
Ex 7-F	488	TGTAAAACGACGGCCAGTGGCTGGGAGTTGCGGAGAAT
Ex 7-R		CAGGAAACAGCTATGACCGCAGTTTCTACTAAATGCATGTTGCTT
Ex 8-F	578	TGTAAAACGACGGCCAGTCAAGTCTTGGTGGATCCAGATCAT
Ex 8-R		CAGGAAACAGCTATGACCCCACTGAACAAGTTGGCCTGC

PCR carried out according to the program showed in table (2). Three microliter of PCR products were separated on 2% agarose gel with a ladder (100bp) and visualized.

Table 2: PCR amplification program for *TP53* exons.

exon	Initial denaturation	denaturation	annealing	Extension	Final extension
ex 5	95°C 5 min 1 cycles	95°C 30 sec 35 cycles	56°C 30 sec 35 cycles	72°C 30 sec 35 cycles	72°C 7 min 1 cycles
ex 6	95°C 5 min 1 cycles	95°C 30 sec 35 cycles	55°C 30 sec 35 cycles	72°C 30 sec 35 cycles	72°C 7 min 1 cycles
ex 7	95°C 5 min 1 cycles	95°C 30 sec 35 cycles	58°C30 sec 35 cycles	72°C 30 sec 35 cycles	72°C 7 min 1 cycles
ex 8	95°C 5 min 1 cycles	95°C 30 sec 35 cycles	53°C 30 sec 35 cycles	72°C 30 sec 35 cycles	72°C 7 min 1 cycles

PCR Products Sequencing.

To detect the mutation in *TP53* Exons, PCR products for each primers were sent to Macrogen company (U.S.A) for sequencing. The sequences of these samples were analyzed by using NCBI site and T-COFFEE program.

3. Results

Demographical Study

This study extend for two years and three months, with an age of volunteers ranged between (14-67) year. In this part of study, the subjects distributed according to the age, gender, consumption

of pack number smoked a day and duration of smoking, as following:

Distribution of smokers according to age

Table 3. Distribution of smokers volunteers according to age groups.

Age groups (year)	Number and percentage of smokers
14-25	19 (12.67%)
26-35	32 (21.33%)
36-45	38 (25.33%)
46-55	32 (21.33%)
More than 56	29 (19.33%)
Total	150
Chi-square-χ^2 P-value	4.982 * 0.0369
* (P<0.05)	

According to age, the subjects were distributed into (5) age groups, the result revealed that the highest number of smokers was located in the third age group (36-45) year which represented 38 (25.33%) of the total, and almost similar number 32 (21.33%) was gained from each (26-35) and (46-55) year age groups. While the lowest number of smokers were at the age groups more than 56 year and (14-25) year which showed 29 (19.33%) and 19 (12.67%) respectively with significant difference (P≤ 0.05) (Table 3).

Distribution of smokers volunteers according to gender

The distribution of smokers according to gender was 91(60.67%) males and 59 (39.33%) females, with the high significant difference (P≤ 0.01) as shown in table (4).

Table 4. Distribution of smokers volunteers according to gender.

Gender	Number and percentage of smokers
Male	91 (60.67%)
Female	59 (39.33%)
Total	150
Chi-square-χ^2 P-value	8.517 ** 0.0073
** (P<0.01).	

Distribution of smokers voluntaries related to consumption of pack number smoking a day

The distribution of heavy smokers volunteers according to consumption of pack number smoked a day, showed the highest number 134 (89.33%) were smoking more than one pack per day against 16 (10.67%) who smoking one pack a day with a high significant difference (P≤ 0.01) (Table 5).

Table 5: Distribution of smokers volunteers related to number of pack consumption per day.

Number of pack consumption per day	Number and percentage of smokers
One pack	16 (10.67%)
More than one pack	134 (89.33%)
Total	150
Chi-square-χ^2 -value	14.067 ** 0.0001
** (P<0.01).	

Distribution of smokers voluntaries related to duration of smoking

The distribution of heavy smokers depending on duration of smoking by years, revealed that the highest number 46 (30.67%) of smokers were smoking for a period of (16-20) year, also nearly a similar number 44 (29.33%) were found smoking for (11-15) year, while 38 smokers (25.33%) smoked more than 20 year and the less number, 22 smokers (14.67%) smoked from 5 to 10 years with a high significant (P≤ 0.01) as described in table (6).

Table 6. Distribution of smokers volunteers related to duration of smoking.

Duration of smoking groups	Number and percentage of smokers
5-10	22 (14.67%)
11-15	44 (29.33%)
16-20	46 (30.67%)
More than 20	38 (25.33%)
Total	150
Chi-square-χ^2 P-value	6.338 ** 0.0146
** (P<0.01).	

DNA extraction

The result of purity was good and ranged from (1.7-1.9) and the concentration ranged from (30.3-45.5 ng/ µl).

whereas The result of gel electrophoresis of genomic DNA showed a clear sharp band as in figure (1), and that suitable for further step and analysis by PCR.

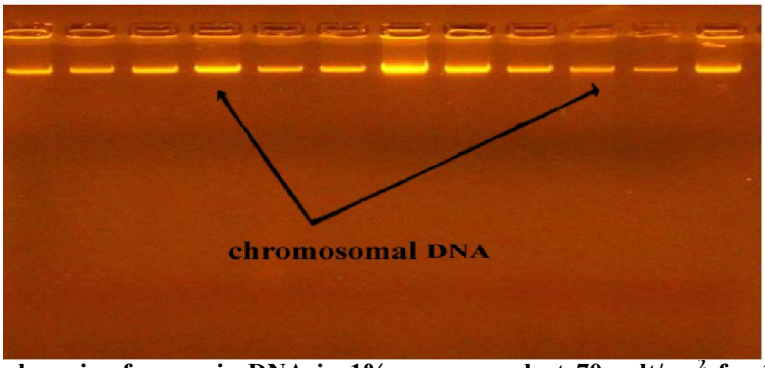

Figure 1. Gel electrophoresis of genomic DNA in 1% agarose gel at 70 volt/cm^2 for 30 min, stained with Ethidium Bromide and visualized under U.V.

Polymerase chain reaction (PCR) analysis

The PCR analysis was employed in this study for amplified *TP53* gene exons (5,6,7 and 8) The result of exon 5 showed an amplified fragment of 588bp as a clear band by electrophoresis on a 2% agarose gel at 70 volt/cm2 for 90 minute as shown in figure (2).

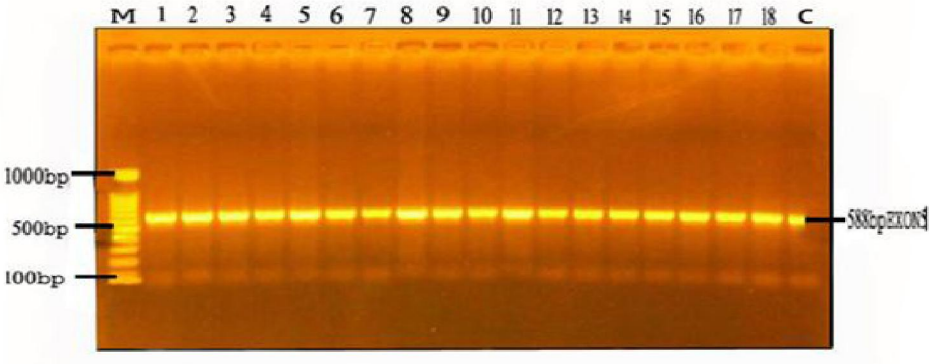

Figure 2: Detection of PCR product of *TP53* gene exon 5 (588bp). The amplified fragments were separated by electrophoresis on a 2%agarose gel, stained with Ethidium Bromide at 70 volt/cm2 90 minute. Photographed under UV light.

M: DNA ladder (100 pb); C: Control.

Lane (1-18) amplified DNA of smokers samples.

While, the result of amplification exon 6 was appeared as a clear bands of 248bp compared with ladder (Figure 3**).** The result of exon 7 showed amplified fragment of 488bp as a clear band by electrophoresis on a 2% agarose gel at 70 volt/cm2 for 90 minute as shown in figure (4**).**

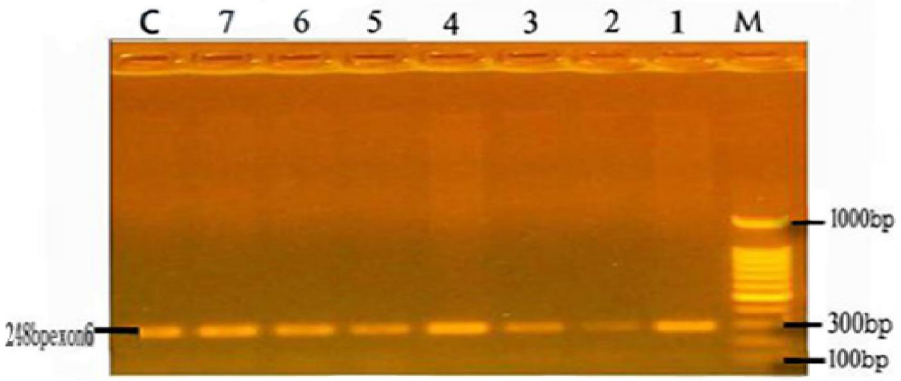

Figure 3. Detection for PCR product of exon 6 gene fragment (248bp). The amplified fragment were separated by electrophoresis on a 2% agarose gel, stained with Ethidium Bromide at 70 volt/cm2 for 90 minute. Photographed under UV light.
M: DNA ladder (100 pb).
C: Control.
Lane (1-7) amplified DNA of smokers samples.

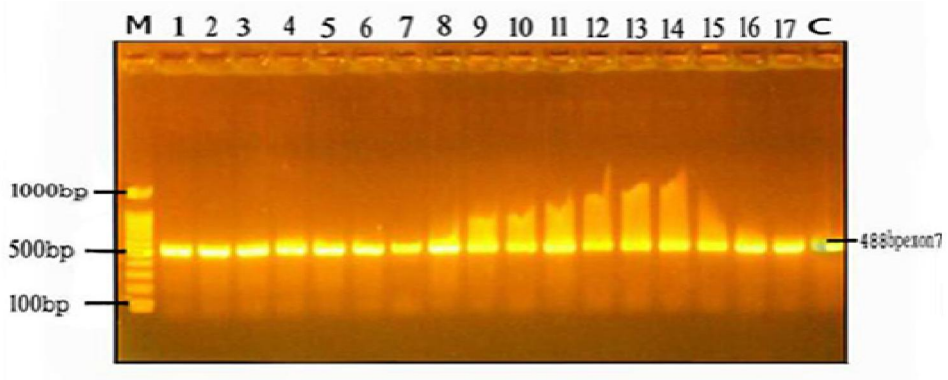

Figure 4. **Detection for PCR product of exon 7 (488 bp). The amplified fragment were separated by electrophoresis on a 2% agarose gel, stained with Ethidium Bromide at 70 volt/cm2 for 90 minute. Photographed under UV light.**
M: DNA ladder (100 pb).
C: Control.
Lane (1-18) amplified DNA of smokers samples.

The result of amplification exon 8 showed as a clear band of 578bp by electrophoresis on a 2% agarose gel at 70 volt/cm2 for 90 minute (Figure 5**).**

Sequencing of *P53* gene

Sequencing results found that a number of the variations on *TP53* gene were characterized by substitution polymorphisms, which assigned as G to C polymorphism as a single nucleotide polymorphism (SNP) in exon 5 at site 338 when Guanine(G) substituted by Cytosine (C) causing missense mutation in the sequences of amino acid Glutamine (CAG) which changed to histiden (CAC) with high number reached to 71(47.3 %) among smokers, in comparison with non- smokers control (0.0%).

On the other hand, the prevalence of deletion mutations had shown in smokers samples, it was observed that exon 6 had deletion mutations among smokers individuals at a number 29 (19.3%) rather than in the non- smokers (0.0%), it was shown Guanine(G) deletion at site 8 which changed the amino acid from Arginine (AGG) to Serine (AGT).

Another deletion of Adenine (A) from Leucine (TTA) at site 16 caused silent mutation that did not cause any variation in the result amino acid, the same

amino acid (Leucine) remained. Beside, the deletion of Adenine (A) occurred at site 18 converted the amino acid from Lysine (AAA) to Asparagine (AAT) (Table 7 and Figure 6); however, no genetic variations were shown in exons 7 and 8 in all samples whether smokers or non-smokers.

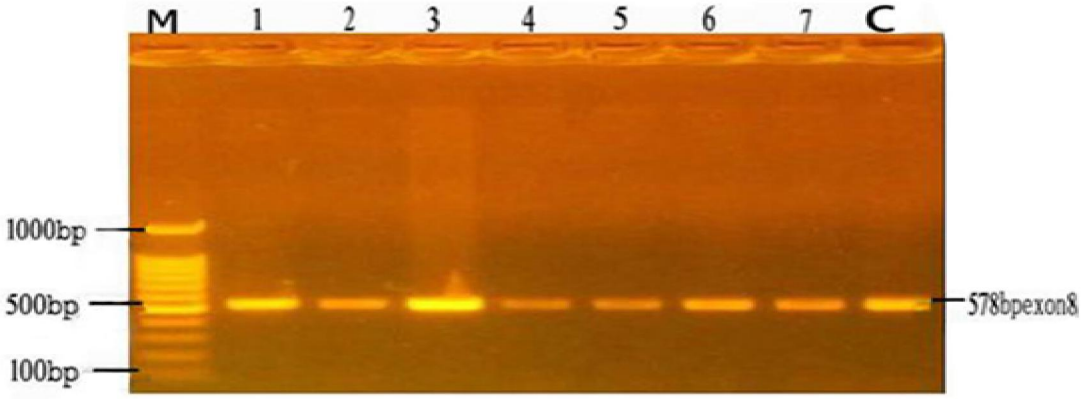

Figure 5.: Detection for PCR product of exon 8 (578bp). The amplified fragment were separated by electrophoresis on a 2% agarose gel, stained with Ethidium Bromide at 70 volt/cm2 90 minute. Photographed under UV light.
M: DNA ladder (100 pb); C: Control.
Lane (1-7) amplified DNA of smokers.

Table 7. Types of mutations of *TP53* gene sequencing

Wild type	Mutant type	Site of mutation	Change in amino acid	Type of mutation	Effect on translation
CAG	CAC	338	Gln - His	Substiution	Missense
AGG	AGT	8	Arg - Ser	Deletion	Frameshift
TTA	TTA	16	Leu - Leu	Deletion	Silent
AAA	AAT	18	Lys - Asn	Deletion	Frameshift

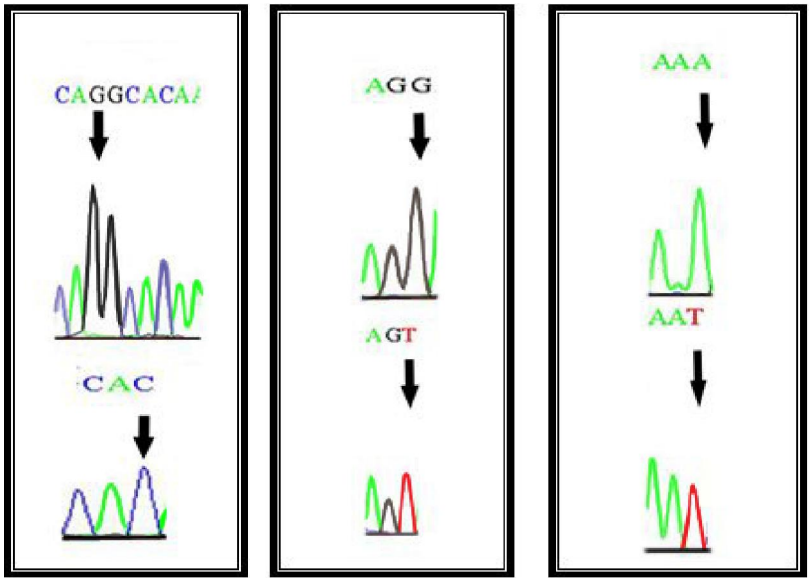

Table 6. Types of mutations of *TP53* gene sequencing

Discussion

The results showed that the highest number of smokers was located in the age groups: (36-45),(26-35) and (46-55) year, this may be due to that the individuals of this three age groups might have seen political events and economic changes in Iraq, which led to the lack of emotional and social stability and, or to that these individuals have salary or other sources of income, resorting to the smoking the cigarettes, A number of studies were done in this field like a study conducted by Moizs *et al*. (2013) who employed out of 1426 people (smokers and non-smokers) who participated in the lung screening program, with average age (54.0±16.3) years, their result showed the highest ratio of smokers was in the age group of 39 years old or younger, followed by smokers in the group of (45-49) years old. Another Another study carried out by Zhu *et al.*(2014) which was examined the smokers behaviors among medical and other college students in China, the results showed that the peak of prevalence (36.4%) was in the age group (21-29) year, and the lowest incidence was in the age group <20 year.

Also, the study revealed that the smoking males were more than smoking females and that may be due to the phenomenon of smoking being socially unacceptable for women in Iraq, in addition, this number may be inaccurate because of the omission or women's reluctance to reveal the reality of being a smoker. In contrast with the current study, some studies showed the female's predominance among smokers, as the study conducted by Campbell *et al*. (2014) which showed that about 43 (60%) of smokers were females and 26 (40%) males, who attended Nova Southeastern University -United States, with the age ranged between (21- 50) years old.

Besides, the study of Colgan *et al*. (2010) found that the majority of smokers were females 55 versus 37 of males out of 92 vocational education students, when they determined the resilience to cigarette smoking among young Australians at risk.

The high number of heavy smokers who smoked more than one packs a day, indicated that most smokers may addicted the nicotine material and may other chemical compound present in cigarette smoke (Benowitz *et al*., 2009), also Belsky *et al*. (2013) attributed that to the fact of smoking addictive chemical substance, especially nicotine, that is difficult to control, leads to the high number of packs consumed by smokers.

In this aspect, there are many studies as that mentioned by John *et al*. (2011) found more than a million-and-a-half smokers between the years 1965 and 2007, when they tracked the prevalence of heavy smokers who smoke 20 cigarettes or more per day in California, whereas the study done in Hungary by Moizs *et al*. (2013) observed that most people, 66.2% of the smokers smoked at least half a pack of cigarettes per day, and 19.1% of the smokers, smoked at least one pack of cigarettes per day, other study of Linnebur (2006) found that Italian men who smoked more than 10 cigarettes per day were significantly higher when investigated emphasizing impotence as a consequence of smoking**.** Furthermore, it was noticed that 90 (60%) of smokers were smoking for 11-20 year and 38 (25%) were smoked for more than 20 year, and when they were asked about giving up smoking, they couldn't due to that the smoking is very addictive because tobacco contains a powerful drug – nicotine - which makes quitting smoking a difficult task. Cigarettes made consciously to give a rapid nicotine hit, it takes less than (20) seconds for the drug to reach a brain from snuffed cigarette smoke. Nicotine leads addiction almost such as heroin or cocaine, it is fully as addictive as these 'stronger' drugs, this is the reason why the most smokers say they want to giving up smoking but they couldn't (Zmeskal *et al*., 2016)**.**

Many studies focusing on duration of smoking as that conducted by Guo and Sa (2015) who found that the higher ratio of the study samples was with duration extended to 20 year, when they investigated the duration of smoking among adult male smokers in China, as well as Jack *et al*. (1990) found that the person who smoked for 40 year was at risk approximately 3.5 times that for a non- smokers, when assessing 752 patients whom suffered from carotid atherosclerosis, and noticed that the total years of cigarette smoking was the most significant independent predictor of the presence of severe carotid atherosclerosis.

In present work, NanoDrop was used to measure the concentration and purity of the DNA. Many studies were used the same apparatus such as study of Hue *et al*. (2012) when extracted human genomic DNA from dried blood spots and hair roots, also the study of Desjardins and Conklin (2010) used NanoDrop to quantitate the nucleic acids.

Moreover, Many researches were investigated the mutation of *TP53* as Greenblatt *et al*. (1994) who found the most rate of *TP53* mutation (87%) were occurred in exons 5–8, and most of the others were in exons 4 (8%) and 10 (4%), when compiled *TP53* mutations in various human tumors, by estimated 560 mutations from more than 300 papers published, in which the entire coding region of *TP*53was sequenced, and Ronchetti *et al*. (2004) analyzed *TP53* gene mutations in exons 5 through 8 by PCR–single-strand conformation polymorphism, when study the association between *TP53* gene mutations and tobacco exposure in 84 patients in laryngeal squamous cell carcinoma, Furthermore, Rozenblum *et al*. (1997)

reported that 76% of all *TP53* mutations occured in exons 2–11 when analyzed 47 cases of respected cancers.

Most early investigators as Levine *et al*. (1991), analyzed *TP*53 chiefly in exons 5–8, which was highly conserved through evolution and presumably of functional importance, 95% of the reported mutations were found in exons 5–8.

Numerous recent studies uesd PCR as a method for the detection of the mutations of *TP*53 gene, as the study conducted by Hosseinrad *et al*. (2016) who used exons 7and 8 to observe the relationship between pulmonary adenocarcinoma and *TP53* tumor suppressor gene mutation, as well the study of Koshino *et al*. (2016) who detected the mutations in exons 5-8 to estimate its correlation with the clinical outcome in lymphoma, whereas Muhartono *et al*. (2016) amplified *TP53* fragment with exons 5-8 to investigate the effects of mucoxin on proliferation, expression of *TP53* gene in various cancer cells.

Likewise, Dastjerdi *et al*. (2016) used these exons when they studied the effect of thymoquinone on *TP53* gene expression and the consequence apoptosis in some cancer cell line. Morevere, Nadhum and his colleagues (2016) investigated the contribution of *TP53* (exons 5 and 6) expression as diagnostic markers for colorectal cancer.

Substitution polymorphisms refers to a change in one amino acid in a protein, arising from a point mutation in a single nucleotide (Watson *et al*., 2008), and can turn the production to non functional protein, which be responsible of body disorders (Minde *et al*., 2012 and Miosge *et al*., 2015).

Pfeifer *et al*. (2002) conducted their study on the *TP53* polymorphism substitution patterns in lung cancers and found difference between smokers and non-smokers with an excess of G transversions in smoking-associated cancers, also the prevalence of G transversions is 30% in smokers' lung cancer but only 12% in lung cancers of non-smokers. It was cleared that the gene *TP53* is the most commonly mutated tumor-suppressor gene in cancer, these mutations are usually widespread in smokers than in nonsmokers (Krishnan *et al*., 2010).

Tobacco exposure produces a heavy load of genomic mutations, including mutation of the tumor suppressor *TP53*, both lack of the wild-type *TP53* function and acquire of mutant (Gibbons *et al*., 2014).

The missense mutant *TP53* alleles show dominant-negative activity through their ability to form p53 tetramers or other protein-protein complexes, and the converted DNA binding of the mutant proteins can get-of-function activity, many of the effects of mutant p53 may be due during its ability to oligomerize to form mixed tetramers with the *p53* family members (Gaiddon, 2001).

As p53 monomers oligomerize, shape the functional tetramers, and cooperate with many other proteins and sequence-specific promoter elements to produce target gene transcription, the occurrence of mutations can alter the protein-protein interactions that define its proper function (Muller and Vousden, 2013).

In present study the polymorphism displays substitution of G → C in sit 338 of the *TP53* gene, changing the amino acid from Glutamine (Gln) to histiden (His) in the Glutamine -rich domain of *P53* protein, however the are molecular differences in *P53* protein structures which form of *p53* Gln and *p53*His (Matés *et al*., 2006), as well as, Glutamine is necessary for nitrogen- induced proliferation in many cells, but glutamine stimulates not only the growth of cells but also many more vital events, among all of them, apoptosis is a recent but prominent incorporation to the whole of phenomena regulated by this distinctive amino acid (Gonzalez Herrera *et al*., 2015).

Apoptotic signalling mechanisms concerned in response to glutamine deprivation are cell type-specific. In any case, new findings indicate that glutamine availability is strongly related to the induction of apoptosis, working both as a nutrient and as a signalling molecule, acting directly or indirectly on the pathways leading to the programmed cell death (Zhdanov *et al*., 2014). So in this study the change of glutamine to histiden may play important role in decreasing the apoptosis.

In addition, and beyond any doubt that *P53* Gln and *p53* His differ in their capability to regulate *TP53*-dependent cell processes, as compared to *P53* His, *P53* Gln protein is the best transactivation molecule (Chen and Cui, 2015) and displays a high capability to block the cell cycle, induce DNA repair (Kron and Bode, 2015), in contrast, *P53* Gln protein induces apoptosis markedly better and with faster kinetics than *P53* His (Gross *et al*., 2014 and Mohamed *et al*., 2014).

On the other hand, DNA adducts stimulate by different mutagens may have significantly various mutational properties, Cheng *et al*. (2003) was investigated the adduct formation between nicotine and DNA. Adducts may form in a base and sequence-specific context, a particular adduct may induce predominantly G → C transversions within a particular sequence context, and if such mutations were found in one type of human tumor, such a carcinogen would become a suspect, in particular if there is epidemiological evidence that exposure to this agent may be involved in causing this type of cancer, and that also confirmed by Hussain *et al*. (2001) who demonstrated that an excess of G→C transversions

was a characteristic of lung cancers related to smoke exposure.

Current result is appeared to be in agreement with many studies in this field as Tiwawechac *et al*. (2003) who studied the relationship between smoking and nasopharyngeal carcinoma (NPC) in Thailand, and revealed the polymorphism of gene *TP53* is influenced by smoking history, Liu *et al*. (2013) also suggested that the *TP53* polymorphism elevated the smoking-related lung cancer risk, indicating that the smoking and gene interact with lung carcinogenesis in a Chinese population, and Vahakangas *et al*. (2001) suggested that the *TP53* mutation's spectrum was arise in active smokers as well as in former smokers, when they study the effects of tobacco carcinogens on inducing mutations at specific codons in *TP53* in human bronchial epithelial cell cultures.

Otherwise converting the amino acid from Arginine (AGG) to Serine (AGT) in the case of Guanine(G) deletion, leads to alter cell capacity to provoke apoptosis, Arginine induces apoptosis mediated through the NO synthase pathway and can inhibited tumour growth (Muller and Vousden, 2014), also protein arginine methyltransferase (PRMT5), as a co-factor in a DNA damage responsive co-activator complex that interacts with *TP53*, is responsible for methylating *TP53*, Arginine methylation is regulated through the *TP53* response and affects the target gene specificity of *TP53* (Jansson *et al* 2008). Furthermore, PRMT5 depletion triggers *TP53*-dependent apoptosis and methylation on arginine residues is an underlying mechanism of control during the *TP53* response. Thus, Arginine plays a major role in inducing apoptosis in *TP53* mutant cells (Schneider-Stock, 2004). Mutations that alter these specific arginine residues in *TP53* have been detected in human cancers (Li and Diehl, 2015).

Also the result of this study represent deletion of Adenine (A) occurred at site 18 that converted the amino acid from Lysine (AAA) to Asparagine (AAT) and led to deviation and changes in the *TP53* function subsequently apoptosis. So, it affected the proliferation of the cells, which may be interferes with the formation of cancers and tumors. Lysine contributed to this process by acetylation of *TP53* which occurred particularly at lysine, a DNA-binding domain residue, and that is essential for triggering apoptosis as mentioned by Sajjad *et al*. (2014). N-terminal acetylation showed that it can guide the localization of proteins. It also been proved to relate with cell cycle regulation and apoptosis leads to the induction of *TP53*-dependent apoptosis. Proteins are typically acetylated on lysine (Kalvik and Arnesen, 2013).

Roos and Kaina (2006) and Hollstein *et al*.(2013) reported that about the half of all cancers examined have a point mutation or deletion in the *TP53* gene and most of these mutations give increase to amino acid changes in evolutionarily conserved sites of the protein, suggesting that they are functionally important changes.

Many studies link the exposure to carcinogens with *TP53* mutations, as that by Puisieux *et al*., (1991) who found 14 mutations, most of which were GC→TA, the researchers suggested that benzo[*a*]pyrene in tobacco smoke specifically causes GC→TA mutations in the *TP53* gene. In esophageal cancer, related to smoking consumption, a wide range of *TP53* mutations had been found, most commonly is GC →AT and GC →TA(Hollstein *et al*.,1991) and Halvorsen *et al*. (2016) analyzed tumors in 394 patients of non-small cell carcinomas, their results demonstrated that *TP53* mutations were identified in 47.2% of the samples, in addition, the frequency of frame shift mutations was (20.3%), they concluded that *TP53* mutation patterns differ among the subgroups of lung cancers, who were influenced by smoking history, also indicated that smoking-induced *TP53* mutations may have a different biological impact than *TP53* mutations occurring in never-smokers.

Liu *et al*. (2014) observed a high percentage of *TP53* mutations reached to (40%) of total, among a heavy smokers when study 1232 patents of small cell lung cancer (SCLC) and non–small cell lung cancers (NSCLC).

Ronchetti *et al*. (2004) found that 24 (28.6%) of all cases were positive to *TP53* gene mutations in 84 patients with laryngeal squamous cell carcinoma, and the frequency of the G transversion was (33%) of the most common type of mutation, furthermore a statistically significant association was found between *TP53* mutations and exposure to tobacco smoke (P =0.001), also their data document that a smoking habit is the only independent variable associated with an increased risk of *TP53* mutations in the laryngeal mucosa.

The study of Le Calvez and colleagues (2005) showed that the relative risk of having a *TP53* mutation in lung cancer was up to 13 times higher in lifetime heavy smokers than in people who never smoked all their lives.

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