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# Heterochromatin Polymorphisms and Chromosomes Damage in Heavy Smoking Men

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**Abstract:**
**Objective:** The goal of the study is to appraise the heterochromatin polymorphism and chromosomal abnormalities associated with chromosome in smoking men.
**Methods:** During a 9-months period (January 2014 to September 2014) a total of 60 heavy smoking men (more than 20 cigarettes daily) together with 60 normal controls were subjected to the present investigation. A study of the variants heterochromatin of chromosomes1, 9 and 16 was performed on lymphocyte culture followed by C-banding from a total of 60 heavy smokers and 60 nonsmoker persons. Constitutive evaluation was based on qualitative method, blood culture, chromosomes harvesting and C-band technique were carry out according to the standard methods.
**Results:** Study indicates smoking men had significantly increased frequency of larger C- band variants on chromosome 1 and 9 as compared with nonsmoking men ($p<0.05$). The frequency of inversions revealed significant differences for variants heterochromatin of chromosome 1, 9 and to less extent 16 between smokers and nonsmokers control group and significant association ($p<0.05$).
**Conclusion:** These results confirm the positive correlation between the amount of heterochromatin on chromosomes 1 and 9 and susceptibility of the smoking men to early development of cancer.
**Keywords:** Polymorphisms, Chromosomes, Heterochromatin, Smoking.

## Introduction

The word heterochromatin is used to indicate those regions of chromosomes that remain condensed through interphase as well as during mitosis. Heterochromatin as the library of different satellite DNA is one of fragment in which it becomes spread and fix in different species genome (Podgornaia 2009). Heterochromatin contains highly repeated fractions of DNA sequences that are not transcribed, these sequences are probably clustered in short tandem repeats at the heterochromatin regions of chromosomes 1,9 and 16 (Sumner 1982), (Thopson & Thopson 2001), (Bulazel et al. 2006).The heterochromatin polymorphisms are inherited in a mendelian manner and it shows a great population and evolutionary stability, though ethnic differences have been reported. However, sex and age-related heterochromatin differences of the autosomes have not been reported yet( (Erdtmann 1982),( Berger et al.1983).

Some studies have reported that variations in heterochromatic regions might have deleterious effects (Halbrecht & Shabtay 1976), (Kimberlig 1977), (Bhasin 2005), (Minocherhomji et al. 2009) .Furthermore, also the roles of Y chromosome constitutive heterochromatin and male infertility have been described in literature (Yasseen & Alkhafaji 2004). Because of the need for more knowledge about the role of smoking on the general health in Iraqi population, the present study was conducted with objective of investigation the effect cigarette smoking on chromosome heteromorphic variations and chromosomes anomalies.

## Materials and Methods

We used modified C -band and G- band developed in our laboratory to reveal and detection of cytogenetic damage in heavy smoker men, in order to reach final conclusion about a possible role of smoking on chromosomes. During a 9 months period (January2014 to September 2014) a total of 60 heavy smoking men (more than 20 cigarettes daily) together with 60 normal control were subjected to the present investigation. A study of the variants heterochromatin of chromosomes 1, 9 and 16 was performed on lymphocyte culture followed by C-banding from a total of 60 heavy smoking men and 60 nonsmoking men. The samples were taken from participant with their consent. The age of smoking men ranged from 28 to 48 years with a means SD of 36.2±6.6. The age of nonsmoking men (controls) ranged from 18 to 48 years with a means SD 31.33±9.07. No drugs were taken by the blood donors for smoking men and control group for at least 3 months prior to sampling. The participants who were with genetic disorder, chronic diseases and infections were excluded from present study. Culture conditions and chromosome cytology that has been published previously (Minocherhomji et al. 2009),(Yasseen & Alkhafaji 2004), (Arrighi et al. 1974).

C-banding technique was carried out by a modification of the method of (Arrighi et al.1974) Freshly prepared slides, 1-3 days old, were placed in a freshly prepared 5% (weight/ volume) saturated aqueous solution of barium hydroxide octahydrate [Ba (OH)2 8H2O] for 5 minutes at 37oC. This was followed by thorough rinsing in distilled water to remove any remaining barium hydroxide that would cause further denaturation. Then, the slides were incubated for one hour in 2 x standard saline citrate (SSC) (0.3M sodium chloride containing 0.03 M trisodium citrate) at 60-65oC followed by a quick rinse in distilled water and staining in 2% (percent volume in

 

volume) Giemsa stain in phosphate buffer saline for one hour. The slides were washed briefly in distilled water and dried before mounting in Depex.

Classification of Polymorphic Variations.

The polymorphic variations in the length of centromeric heterochromatin on the long arms of chromosomes 1, 9 and 16 were designated as 1qh+, 9qh+ and 16qh+ (increased heterochromatin). Heterochromatin can also be reduced in these chromosomes, such as 1qh-, 9qh- and 16qh- . The pericentric inversion of chromosomes 1, 9 and 16 preformed directly from a positive photograph using the linear measurement method that has been suggested elsewhere. (Balicek et al. 1978), (Podugolnikova et al. 1979) the absolute C-band length measurements are presented in micrometer and the relative length measurements as percentage. Statistical analyses were performed using the SPSS software package (revision 20 Inc., Chicago,USA) Data are expressed as means ±SD. Differences in distribution of constitutive heterochromatic variants between smokers and nonsmokers were tested using the Chi- square statistic. Odds ratios (ORs) and 95% confidence intervals (95% CI) were calculated to estimate the risk and association of smoking with heteromorphic variants of chromosome. Values of $P < 0.05$ were considered statistically significant

**Results**

**(Table 1)The lengths Variants distribution of constitutive heterochromatin on chromosomes 1,9 and 16 on heavy smoking men and nonsmoking men ( control).**

| Chromosomes with C- band of qh+/qh- | Heavy smokers n =60 | Control n= 60 | OR | (95%CI) | P Value |
|---|---|---|---|---|---|
| Chromosome 1 | 54 (45%) | 32(26%) | 2.3 | 1.3--3.8 | 0.003 |
| Chromosome 9 | 44 (36%) | 18(15%) | 3.3 | 1.7-6.1 | 0.0002 |
| Chromosome 16 | 24 (20%) | 16(13.4) | 1.6 | 0.81-3.2 | 0.16 |

**(Table 2)The Distribution of Pericentric inversion and of constitutive heterochromatin on chromosomes 1, 9 and 16 of heavy smoking men and nonsmoking men ( control).**

| Chromosomes with C- band Inversion | Heavy smokers n =60 | Control n= 60 | OR | (95%CI) | P Value |
|---|---|---|---|---|---|
| Chromosome 1 | 62 (51%) | 30 (25%) | 3.2 | 1.8-5.5 | 0.0001 |
| Chromosome 9 | 16 (13.4%) | 6 (5%) | 2.9 | 1.1-7.7 | 0.03 |
| Chromosome 16 | 12 (10%) | 10 (8.4%) | 1.2 | 0.5-2.9 | 0.6 |

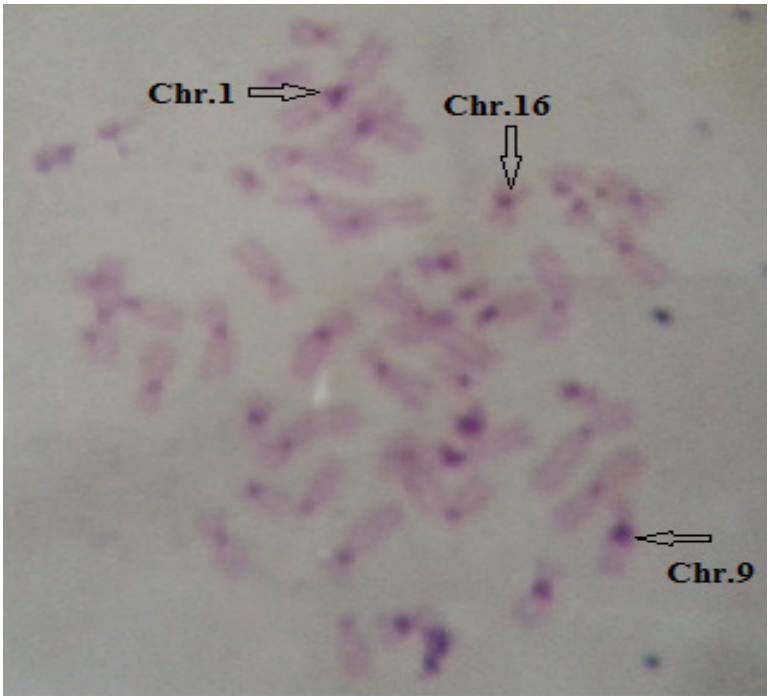


**Fig.1: C-banded metaphase spread of smoking men with normal chromosome constitution 46XY, note the centromeric heterochromatin (C-band) on chromosome 1, 9 and 16. (See Arrowed)**

 

**Results**

The data of our results revealed that the majority of participants showed (46, XY) male mitotic Karyotype with no obvious chromosomal aberration could be detected, in heterochromatins polymorphisms, the study reveals the proportion and analysis chromosomal constitutive heterochromatins polymorphisms in chromosomes 1,9 and 16 and frequency of inversion in heavy smoking men and nonsmoking men (control group). The hetromorphic frequency of constitutive heterochromatin variants in smoking men was 45% for chromosome 1 , 36% for chromosome 9 and 20% for chromosome 16 respectively, whereas in control group chromosome 1,9 and 16 variants were at frequency of 26%, 15% and 13.4% respectively (Table 1). We found a significant difference in size variants on chromosomes 1,9 and 16 between the smoking and non smoking men. Smoking men had significantly increased frequency of larger C- band variants on chromosome 1 ( OR 2.3, 95% CI= 1.3-3.8 P= 0.003), chromosome 9 ( OR 3.3, 95% CI= 1.7- 6.1 P= 0.0002), chromosome 16 the results revealed modrate assciation but it was not significant( OR 1.6, 95% CI= 0.81- 3.2 P= 0.16) as compared with non smoker men. The constitutive heterochromatin inversions frequency obtained in the smoking men was 51% for chromosome 1 , 13.4% for chromosome 9 and 10% for chromosome 16 respectively,whereas in control group chromosome 1,9 and 16 variants were at frequency of 25%, 5% and 8.4% respectively(Table 2). The frequency of inversions revealed significant differences for variants heterochromatin of chromosome 1 between smokers and non smokers control group and significant association(OR 3.2, 95% CI= 1.8-3.2P= 0.0001).Also the defferences and association were highly significant for chromosome 9 of smoking men when compared to non smoking men (control group)( OR 2.9, 95% CI= 1.1- 7.7 P= 0.03). or chromosome 16 the results revealed modrate assciation but it was not significant( OR 1.2, 95% CI= 0.5- 2.9 P= 0.6)

**Discussion**

Polymorphisms of the size of heterochromatin regions of chromosomes have been studied previously (Sumner 1982), (Berger et al. 1985). Our results which conducted with heavy smoking men have shown that heterochromatin variants in chromosomes 1and 9 were higher in smoking men as compared to nonsmoking men (control group). Indeed the study revealed increase in size polymorphisms of chromosome 1 and 9.

In fact, the data from previous studies have observed that the size heteromorphism of constitutive heterochromatin region of chromosome 1 may predispose to malignant disease. This was first showed by Atkin et al. in carcinoma of ovary and cervix (Atkins 1977), (Atkin & Brito-Babapulla 1985). Similar association for size heteromorphism of chromosome 1, 9 and/ or 16 have also been reported for breast cancer (Berger et al. 1985) . Benign and malignant colorectal tumors (Shabtai et al. 1985) and various of hematological malignancies. Furthermore, present investigation revealed a significant high frequency of inversion in smoking men than in nonsmoking men. This result is in full agreement with( Berger et al. 1985) and (Movafagh 2003) who demonstrated high frequency of constitutive heterochromatin inversion in patients with malignancy . In contrast (LeConiant et al. 1985) who did not found increase in frequency of inversion between group of malignancy and control group. Cigarette smoking may cause heterochromatin variants. Tobacco smoke contains many chemicals which are known to cause damage DNA. DNA damage can cause cells to mutate and grow uncontrollably, and can start the body on the route to Cancer (Lawrence et al. 1984), United state Report of the Surgeon General (2014).

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