

Tobacco Addiction: Effect on Human Health

Chapter 5

Tobacco Use and Its Genotoxic Effects in Pregnancy

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Abstract

The current chapter discusses recent findings in humans on genotoxic effects on the fetus of prenatal exposure to smoke tobacco. Tobacco Smoking is the most widespread substance dependence in the World, and is a rapidly increased including in pregnant women and serious public health problem worldwide. Nicotine readily crosses the placenta and the fetuses of mothers. Recent studies have suggested a direct contribution of nicotine the addictive component of tobacco and tobacco smoke to human carcinogenesis, and it remains the most common harmful substance to which pregnant women are exposed. Smoking during pregnancy increases maternal health, and also it has deleterious effects on the fetus; premature birth, intrauterine fetal death, intrauterine growth retardation and congenital anomalies. It is known that cigarette smoking has genotoxic effects and causes mutations. The newborns of smoking mothers have elevated frequencies of chromosome damages and DNA strand breaks. Our results also strongly suggest that nicotine is hazardous to the human fetal cells and adult cells. The toxic substances from cigarette smoke induce chromosomal aberrations (CAs) in vitro and could potentially increase levels of aneuploidy in the fetus. This possibility is consistent with the genotoxic effects in fetal cells from smoking during pregnancy are most likely caused by cigarette constituents, providing a potential mechanism for polyploidies and aneuploidies in fetal cells or embryo. Moreover, increased levels of aneusomy in fetus are correlated with low implantation rates, spontaneous abortions and fetal losses. Due to the harmful effects of cigarette, pregnancy is one of the ideal times to quit smoking. Because, mothers should

repeatedly be aware about the harmful effects of cigarette on their baby health. They must be achieved for quitting smoking before pregnancy, and stop smoking especially if you can get pregnant or no pregnancy before you quit smoking. Likewise, healthcare units and maternity wards should hold lectures and explain the harm of tobacco to health and the environment.

1. Cigarette Smoking Habits and Turkey

The use of narcotic stimulants and the habit of these substances are very important for human and community health. It is known that cigarette and tobacco derivatives are addictive, and is one of the most important threats to human health. Smoking is the most widespread substance dependence in the world. Smoking habit is a complex illness that is not clear the genetic inheritance model and causes many permanent illnesses without recycling. Addiction to tobacco has significant psychosocial aspects, but the basis of physical addiction is nicotine. Tobacco use kills more than 7 million people each year in the world, and was the second leading cause of deaths in 2017 [1]. If the consumption of cigarettes continues this way it is envisaged that around 10 million people will die in 2025 due to cigarette smoking worldwide, 70% of which will be seen in developing countries [2,3].

Smoking is one of the major health problems in Turkey as well as the problem of many developed and developing countries in the World [4]. The acute toxicity of nicotine and the longer-term exposure has adverse effects on reproductive health, lung growth and development, neurocognitive function and cognitive decline, psychiatric morbidity, immune function, cancer risk, and cardiovascular disease. In recent years, however, some important steps have been taken in the social field to combat smoking. Nevertheless, Turkey still ranks 11th in the ranking of most cigarette consuming countries. In Turkey, close to 15 million people (27.1%) (10.6 million men and 3.9 million women) currently smoking every day, and cigarette consumption has increased by 52% over the last 10 years. At the same time, 5 million people are also exposed to cigarette smoke. The frequency of tobacco use is higher in males (41.5%) than in females (13.1%). Among tobacco users, 23.8% use daily tobacco (37.3% of men, 10.7% of women). The majority of users of tobacco products (94.8%) are cigarette smokers and only 0.8% use narghile. About half of the smokers (42.1%) also drink the first cigarette in the first 30 minutes after awakening. The average age at everyday cigarette smokers is 17.1 years and most of the smokers (58.7%) have started smoking before the 18th year of legal age[5].

According to the results of the WHO in 2014, passive smoking appeared in 39% of the working population in Turkey. Unfortunately, 55% of our children are exposed to passive smoking by their families. Over 1985-2000 period, cigarette consumption in Turkey has increased by 89% [6]. This shows that despite the smoking ban, the rate of passive smoking is increased to 58% in closed areas. There are 1 million women, 4 million men and about 5 million passive smokers who are exposed to cigarette smoke while they are not smoking at

work in Turkey. Passive smoking is particularly threatening the babies and born babies in the womb. This reduces the chances of a healthy individual in the future. This shows that passive smoking is effective and bad. In Turkey, 120 thousand each year and 300 people every day die due to smoking reasons.

If the current smoking situation continues this way, in 2020, about 10 million people die each year in the world because of smoking and 7 million of them will be from developing countries. For this reason, the WHO considers reducing tobacco use and tobacco-related deaths as a top priority [7]. According to the survey results, when compared with 2008 and 2012, smoking rates in Turkey has regressed from 31.2% to 27.1%. This rate decreased from 47.9% to 41.5% for males and from 15.2% to 13.1% for females. The rate of those who started smoking before the age of 15 declined from 19.6% to 16.1%. The most significant decline in the period of 2008 - 2012 has been seen with regard to passive smoking, and it is noteworthy that the rate of smoking in restaurants has decreased from 55.9% to 12.9%. In Turkey, the National Tobacco Control Program by applying determination, MPOWER became the first and only country to fulfill all of the strategies in the policy package [3].

2. How Many Women Smoke During Pregnancy?

Smoking habits among women starting in adolescence can turn into physiological and psychological dependence when it comes to women's marriage. Smoking is the greatest danger in pregnant women; a major health problem because of the damage the fetus gives to pregnant women. Nevertheless, these increases in cigarettes are more common among young people and women, which cigarette companies regard as the target audience. At the same time, especially these companies are trying to increase smoking rates among women by giving messages that smokers look more attractive or more modern, and also encourage cigarette smoking in adolescence, a period when women may feel the most need for themes such as freedom and power. In this period, women's smoking addiction can continue during pregnancy.

Today, it is understood that smoking is an illness and that these people should be treated. Although, cigarette addicts think so because they feel relieved by cigarettes, feel relieved of their stress and are relieved only by taking nicotine. Smoking addiction that develops in women can often continue during pregnancy, and 50-70% of women with cigarette addiction continue to smoke during pregnancy. In a large-scale study conducted, it was determined that approximately one in five women smoked while pregnant. In Turkey, one of the four or five females generally smokes during pregnancy. Smoking in pregnancy is extremely harmful to both mother and baby health. We know that maternal smoking and smoke exposure during pregnancy are detrimental to fetal growth and development. In 2002, about 11.4% of all pregnant women in the USA were using cigarettes[8], and maternal cigarette tobacco smoking is the leading cause of premature morbidity and mortality in the United States [9]. The prevalence

of tobacco use during pregnancy was found to be in 2012, 20.5% in United Kingdom, in 2012, 11.2% in the 2001-2006 in Canada, 69% in pregnant women-Australiannatives (aborigines) and 17.2% in Nepal [10-13]. The prevalence of waterpipe smoking among pregnant women is around 6% in Lebanon [14] and 9% in Jordan [15]. According to WHO date (2005), 22% of women in developed countries are reported to be smoking [16]. Similarly, in our country which is in the status of a developing country, it is known that the number of women smoking increased over the years and that about one in four women smokes.

Smoking or being exposed to smoke, the most extreme example of a systemic human mutagen, is the most important preventable cause of diseases or deaths. Despite these damages of nikotin it is estimated that about 20–27% of women still smoke during pregnancy [17]. Increased cigarette consumption in a society can also increase the risks associated with smoking-related pregnancy. The adverse effect of smoking during pregnancy can be also caused by cigarette smoke in the environment as well as from smoking in pregnant women. Environmental cigarette smoke is known to be a common negative factor for pregnant women. There is not enough number of studies in pregnant women who report smoking status in Turkey. Only, there are few studies on the harms of cigarettes in pregnancy. In a study carried out in Turkey, the percentage of women who had smoked at any time during pregnancy was 17%. The percentage of women who smoked throughout pregnancy period was 9%. The prevalence of current smoking among them on the first day after birth was also 9%. The percentage of current smokers among the husbands of the respondents found as 68% [18]. According to a report prepared by Turkey Statistical Institute in 2012, smoking rate was 41.4% for males and 13.1% for females. At the same time, 11% of pregnant women and 17% of breastfeeding mothers are smoking cigarette [5]. In Turkey, the studies on smoking before pregnancy in women was observed in the range of 35.3%-13,7% rate of smoking¹⁻⁶, and the rate of smoking in pregnancy was found to be 11.9% [3].

3. Smoking During Pregnancy Harms the Genetic Structure of Mother and Baby

Tobacco smoking and smoke exposure during pregnancy seriously damages both mother and baby health and cause considerable childhood morbidity and mortality. Tobacco smoke is the most extreme example of a systemic human mutagen. Because, normally nicotine passes to fetus, placenta, amniotic fluid and milk of smoking mothers [19]. Thus, maternal smoking continues to be a leading preventable cause of pregnancy complications in otherwise low-risk women. Smoking harms the heart, veins and all other organs of the body, especially the respiratory system. These harmful effects of cigarette are seen in two ways, short and long term. Nicotine and carbon monoxide in the cigarette are extremely harmful to the baby. The baby is fed through placenta and cord. In smokers' mothers, babies can not feed enough and can not develop because they can not carry enough oxygen to the baby. Oxygen is the most important means for the growth and development of infants. When oxygen is reduced in the

mother's blood, the amount of oxygen the baby receives and the amount of nutrients are also decreasing. Therefore, the development of growth and development in the infants of smokers' mothers can occur. When the cigarette is left in the first trimester (the period up to the 12th week of pregnancy), the fetus in the mother's womb is less damaged than in the other periods [20,21]. Approximately one third of infants are exposed to environmental tobacco smoke. Smoking is among the most important preventable causes of intrauterine, infant and childhood diseases and deaths; including low birth weight, premature delivery, spontaneous abortion, placental abruption, perinatal mortality and ectopic pregnancy [22-24]. For this reason, smoking cessation in pregnancy is extremely important for mother and baby health. Although knowledge about the negative effects on the fetus and the newborn of smoking during pregnancy is getting increasingly widespread, this habit still remains a great problem worldwide. The International Child Care Practices Study concluded in a survey of 21 centers in 17 countries that an average of 22% of mothers and 45% of fathers were smoking at the time of their child's birth [25].

Recent studies have suggested a direct contribution of nicotine the addictive component of tobacco to human carcinogenesis, and it remains the most common harmful substance to which pregnant women are exposed. Most people know that smoking causes cancer, heart disease, and other major health problems. The effects of smoking for fetus during pregnancy depend on chemical materials of contents. Nicotine, carbon monoxide and cadmium are among the most important ones. The main harmful substance for fetus is acetaldehyde. At the same time, tobacco smoking during pregnancy has been reported as one of the source of oxidant status [26]. Cigarette smoking can lead to oxidative stress for smokers and those exposed to smoking, as well as reduce the level of certain antioxidants. It has been clearly demonstrated that these harmful substances are a potent inducer of DNA strand breaks in human and rodent cells [27-30]. Mutants and free radicals in cigarette are known to cause DNA strand breaks during DNA synthesis and thus block DNA synthesis. DNA damage has even been observed in groups that consist of young populations (19 to 23 years old) with a brief history of smoking [31]. Studies have found that there is a relationship between prenatal environmental smoke exposure and neonatal DNA damage [32]. However, knowledge about the possible genetic effects of prenatal nicotine exposure in humans is to date limited. It has been shown that the exposure to tobacco increases the potential for chromosome breakage at some cancer sites in the genome. In a study, we showed that smokers had a higher frequency of total CA expression compared with non-smokers [33]. Various studies have also found that smoking caused a 10–20% increase in CAs frequency [34], and in vitro exposure of peripheral lymphocytes to smoke, results in higher CAs frequencies [35-38]. A similar study also show a significant increase in the CA frequency in smokers when compared to non-smokers [39]; the incidence of CAs was between 8.1 and 54.2% with a mean of 26.5%.

In an our similar study, the smoking group exhibited a higher frequency of total CA

expression compared with that of the non-smoking control group ($P < 0.001$), but except for 1p36, 3q21 and 5p15 regions, the overall difference was not statistically significant ($P > 0.05$) [33]. In the smoking group, FS at 1p36, 3q21 and 5p15 regions were significantly increased. It is interesting that the tobacco compounds were particularly interactive with cancer loci but not with the other loci. It has been noted in studies conducted by other researchers that these three regions are potential sites for both the development of some cancers and the development of smoking habits. These regions may be evidence for a common genetic factor that contributes to smoking. This also shows that the exposure to tobacco increases the potential for chromosome breakage at three cancer sites in the genome. At the same time, there is a relationship between the cancering materials and the cancer gene-regions. What are the molecular mechanisms that provide attraction between these dangerous regions and the carcinogenic substances in cigarettes? Its molecular mechanism is unknown. The other studies demonstrated that a significant increase was found in the frequency of CA and FS between smokers and nonsmokers [39-41].

These observations should stimulate more studies on these chromosomal regions at the molecular, cytogenetic, and population genetic level. These three regions have been previously identified as potential susceptibility loci for several cancers and may have susceptibility loci that are specific for the development of habitual smoking. The 1p36 band is a cancer breakpoint [42]. Reciprocal translocations between 3q21 and other chromosomes are well documented in myelodysplastic syndrome and leukemia [43,44]. Previous studies have shown that the 5p15 region exhibits frequent genetic changes in bronchial epithelial cells in long-term smokers, and in invasive cervical carcinoma, and that these changes arise early during carcinogenesis [45,46]. It is interesting that the tobacco compounds were particularly interactive with cancer loci but not with the other loci. Knowing these mechanisms is very important in terms of prevention of cancer. After that, it will need to work on resolving this relationship.

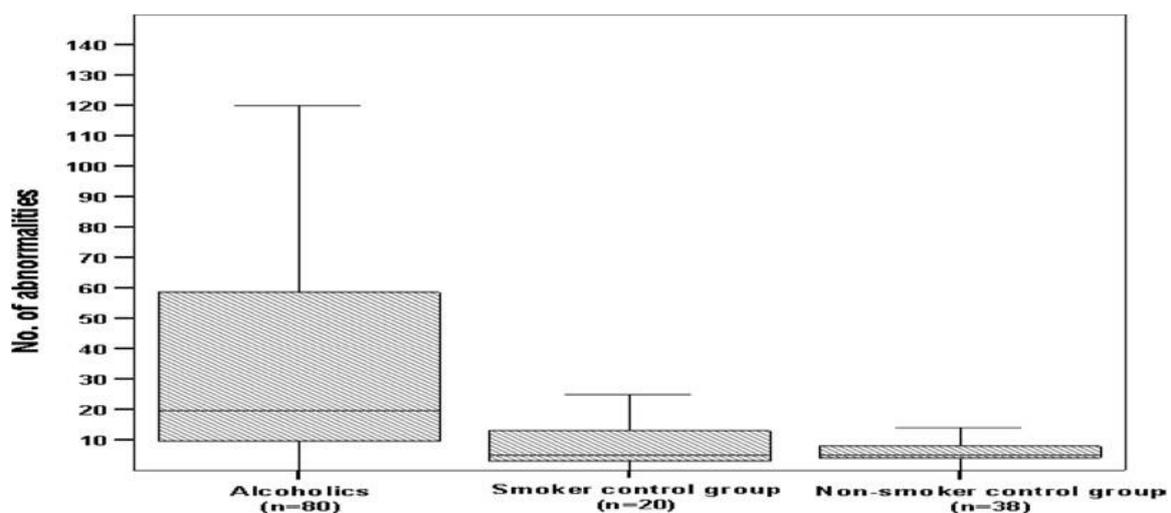


Figure 1. Distribution of chromosomal aberrations in the alcoholic, smoking and non-smoking groups [33].

At the same time, nicotine also affects the cell development of fetus. We personally observed that women who smoked during pregnancy were adversely affected by fetal cell development in routine amniotic cell cultures. The growth of fetal cells in pregnant smokers is later than non-smokers in amniocentesis cultures. According to our findings, there is a significant difference of CAs between nicotine containing medium grown cells and control medium grown cells. Also, this shows that the nicotine passed from mother to fetus it decelerates the development of the baby's cells. Although, information on the *in vivo* genotoxicity of nicotine is limited, in our study it was confirmed that the nicotine leads to significant direct genotoxic effects on human fetal cells *in vitro* [47]. The determination of tobacco-specific metabolites in fetal blood and amniotic fluid also supports the possible genotoxic effects of smoking during pregnancy. Meanwhile, some genotoxicity studies [48,49] have demonstrated the existence of an increased incidence of micronuclei and CAs, as well as sister chromatid exchanges in smoking adults; however, only limited data is available on the possible genotoxic risks of smoking on fetuses and newborns.

High DNA damage is also known to influence apoptosis and cell cycle [50]. Most cells have the ability to perform DNA repair. However, if DNA lesions are mis-repaired and the physiological pathway of apoptosis is interrupted, DNA lesions can cause CAs or other changes with the potential of inducing mutagenesis in a multistep mechanism [51]. Certain studies have shown that nicotine induces aneuploidy and polyploidy [52], SCE and CAs in bone-marrow cells of mice [53]. In a similar our study, there are significant differences in the frequency of CAs between medium containing nicotine and control medium ($P < 0.001$). This data indicates that nicotine expresses significant direct genotoxic effects in fetal cells *in vitro* [46]. However, the causal relationship between smoking in pregnancy, the induction of genotoxic effects of nicotine, and the frequency of embryonal CAs have not been fully documented. The chromosomal breaks and other damage observed in our study may be related to the proliferation of DNA.

In our related study, approximately 20% of all cells were carriers of numerical CAs (total CAs 22.1%) (ure 2). Tetraploidy and aneuploidies were found to be the most frequent abnormalities. Other studies show a significant increase in the CA frequency in smokers when compared to non-smokers; the incidence of CAs was between 8.1 and 54.2% with a mean of 26.5% [46]. These findings may explain the increased aneuploidy rates in fetal cells, and were in agreement with other cytogenetic studies among smokers [36,37]. The prenatal exposure to nicotine increases the frequencies of premature centromere separation and premature anaphase, in agreement with the results of our study which suggested that nicotine elevates aneuploidy levels in human fetal cells. This data indicates that nicotine expresses significant direct genotoxic effects on human fetal-cells *in vitro*. This possibility is consistent with the genotoxic effects in fetal cells from smoking during pregnancy are most likely caused providing

a potential mechanism for polyploidies and aneuploidies in fetal cells or embryo. Accordingly, in a few in vivo studies measuring CAs, nicotine has been reported to interfere with oocyte maturation and chromosome disjunction [51], and to induce aneuploidy and polyploidy in mouse bone-marrow cells [54]. The origin of autosomal trisomies has also been investigated, and several studies showed that smoking can be a confounding factor when assessing aneuploidy and DNA damage in epidemiological studies [55]. In utero exposure to tobacco smoke also increases CAs frequencies in the newborns [56]. Various studies have found that smoking caused a 10–20% increase in CAs frequency [57]. All chromosome groups are represented in aneuploidies, but non-disjunction is not a random event in female meiosis. In particular, there is a significant excess of nondisjunction in the acrocentric D (13, 14, 15. chromosomes) and G (21, 22 chromosomes) groups chromosomes [58] or in the G group chromosomes only [59].

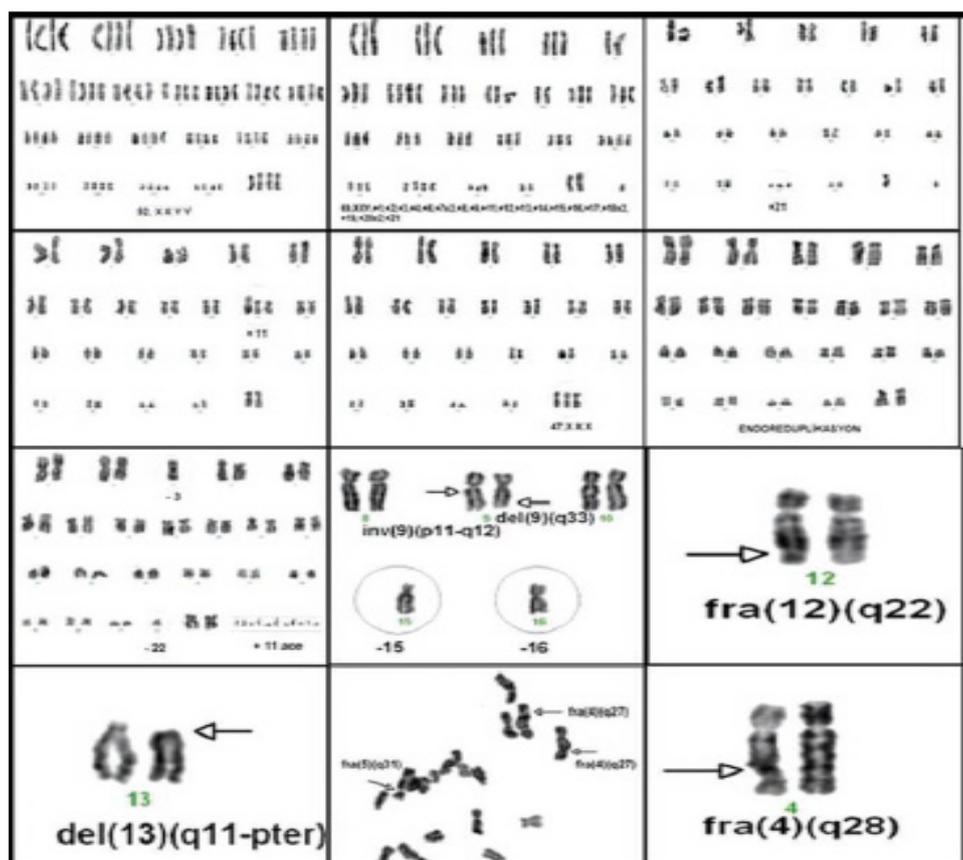


Figure 2: Metaphase and partial metaphase figures showing some chromosomal abnormalities of fetal cells, cultured in medium containing nicotine [46].

This fact has been reported in studies conducted on spontaneously aborted fetuses and live-born infants. In our work on the genotoxic effects of nicotine in smoking mothers; the most common numerical aberrations were chromosome 21 aneuploidies (in 1.7% of all cells and 9% of numerical aberrations), followed by monosomies and trisomies 22, X, 8, 10, 15 and 20, respectively [46]. In particular, there is a significant excess of nondisjunction in the acrocentric G group in our study. This finding shows that G group chromosomes are more sensitive to nicotine in terms of non-disjunction events. Our findings indicate that smoking can be a confounding factor when assessing aneuploidy and tetraploidy in human fetal cells. We speculate that there is an association between prenatal exposure to cigarette smoke and in utero

aneuploidies. Results of this study confirm that the nicotine leads to significant direct genotoxic effects in human fetal cells in vitro, and there is an association between prenatal exposure to cigarette smoke and in utero aneuploidies. Despite the damages explained above it is estimated that about 20–25% of women still smoke during pregnancy [60]. Although knowledge about the negative effects on the fetus and the newborn of smoking during pregnancy is getting increasingly widespread, this habit still remains a great problem worldwide. The widespread use of cigarettes among pregnant women or pre- and post-pregnancy women is a threat to the health of future generations and suggests that these studies should be more active.

We speculate that the toxic substances from cigarette induce structural and numerical CAs in vitro and could potentially increase levels of aneuploidy in the fetus. These findings may explain the increased aneuploidy rates in fetal cells and oocytes of mother, and were in agreement with other cytogenetic studies among smokers [36,37]. Therefore, nicotine could express significant direct genotoxic effects in human cells. This possibility is consistent with the genotoxic effects in fetal cells from maternal smoking during pregnancy, and are providing a potential mechanism for polyploidies and aneuploidies in fetal cells. Just as in a few in vivo studies measuring CAs, nicotine has been reported to interfere with oocyte maturation and chromosome disjunction [53], and to induce aneuploidy and polyploidy in mouse bone-marrow cells [61].

Aneuploidy is one of the most important reason of reproductive biology and reproductive diseases. The origin of autosomal trisomies (13, 18 and 21 chromosomes) has also been investigated, and several studies showed that smoking can be a confounding factor when assessing aneuploidy and DNA damage in epidemiological studies [62]. At the same time, high doses of nicotine increased the frequencies of premature centromere separation and of premature anaphase and reduced the number of oocytes ovulated. Also, it has been suggested that the chromatids arose from premature centromere division at meiosis and it was a major mechanism for the generation of trisomy [63]. Together, these findings indicate that smoking can be a confounding factor when assessing chromosome disjunction (aneuploidy and tetraploidy) in human fetal cells. The human genome is delicately balanced, and for the most part perturbations in the chromosome complement are often incompatible with embryonic development. In particular, there is a significant excess of nondisjunction in the acrocentric G group in our study. This finding shows that G group chromosomes are more sensitive to nicotine in terms of non-disjunction events. For the acrocentric chromosomes 15 and 21, meiosis I errors are the predominant maternal errors, in contrast, for trisomy 18 meiosis II errors predominate. These results strongly indicate that cigarette smoking is hazardous to the viability and function of developing oocytes and their resulting embryos [64].

At the same time, aneuploidy is a well recognised feature of human tumours, and there is a significant correlation between aneuploidy and melanoma thickness. We observed aneuploid

not only in gestation but also in different types of cancer [65,66]. Because, aneuploidy is a well recognised feature of human tumours, and has been proposed to drive tumor development by enhancing genomic instability. The increased incidence of aneuploidy, could contribute to the progression of the disease along with other CAs. Many women are exposed to cigarette smoke. Therefore, the aneuploidy screening is important in pregnancy.

In our study, the numerical changes of sex chromosomes were present in four fetal cells including 47XXX, 45,X[2] and 47,XXY [46]. Smoking appears to induce aneuploidy in sperm for chromosomes 1, 13, and YY disomies, but not for XY, XX, or 7[67-69]. However, sperms of smokers display elevated levels of meiosis II non-disjunction of the sex chromosomes, relative to that of non-smokers [70]. In another our study, the numerical changes of sex chromosomes were present in the maternal cells [64]. The results of our work showing there is a positive correlation between the frequency of aneuploidy and the smoking. Smoking in women may increase sex aneuploidy rates, providing a potential mechanism for aneuploidy in fetal cells or and their resulting embryos. However, sperms of smokers display elevated levels of meiosis II non-disjunction of the sex chromosomes, relative to that of non-smokers [46]. The toxic substances from cigarette exposure induce CAs in vitro and could potentially increase levels of aneuploidy in the fetus.

Some studies found that smoking produced a marginal increase in translocation frequency [71], or caused a significant increase in stable structural aberrations (translocations and insertions) [72]. Also, structural changes were observed in 2.1% of the cells and in 9.7% of the maternal cells with CA in our study. The findings in our last study confirm that the newborns of smoking mothers have elevated frequencies of chromosome translocations and DNA strand breaks. It is known that cigarette smoking has genotoxic effects and causes mutations. There is a positive correlation between the frequency of aneuploidy and the effect of nicotine. Smoking during pregnancy increases maternal health risks as well as mental and physical problems for the fetus, contributing to multiple adverse outcomes such as preterm delivery and stillbirth. It is well understood that the fetal environment is of tremendous importance during the developmental period in determining health throughout the life of the individual [46].

4. Conclusion

Smoking in women is a rapidly growing and serious public health problem worldwide. Tobacco smoking is a risk factor for numerous disorders, including cancers affecting organs outside the respiratory tract. Nicotine readily crosses the placenta and the fetuses of mothers and are exposed to relatively higher nicotine concentrations than their mothers. Also, it has deleterious effects on the fetus. The results of studies strongly suggest that the newborns of smoking mothers have elevated frequencies of chromosome translocations, DNA strand breaks and numerical chromosomal irregularities. It is known that cigarette smoking has genotoxic

effects and causes mutations. The toxic substances from cigarette smoke induce structural and numerical CAs and could potentially increase levels of aneuploidy in the fetus. Due to the harmful effects of cigarette, pregnancy is one of the ideal times to quit smoking. We can certainly conclude that quitting smoking early on in the pregnancy may avoid genetic effects on the newborn. From a public health perspective, it is essential that pregnant women should be advised to give up smoking from conception and avoid exposure to environmental tobacco smoke during pregnancy. Likewise, healthcare units and maternity wards should hold lectures and explain the harm of tobacco to health and the environment. In pregnancy, by determining the factors effecting smoking status, smoking during pregnancy and passive smoking can be reduced through counselling and education services. Thus health care providers and policy makers need to give special attention in those issues and effective implementation of national guideline for effective curving tobacco consumption epidemic during pregnancy. By this way, the effects of smoking on mother, baby and pregnancy can be reduced.

5. References

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