X Chromosome Inactivation in Opioid Addicted Women

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A B S T R A C T

Introduction: X chromosome inactivation (XCI) is a process during which one of the two X chromosomes in female human is silenced leading to equal gene expression with males who have only one X chromosome. Here we have investigated XCI ratio in females with opioid addiction to see whether XCI skewness in women could be a risk factor for opioid addiction.

Methods: 30 adult females meeting DSM IV criteria for opioid addiction and 30 control females with no known history of addiction were included in the study. Digested and undigested DNA samples which were extracted from blood were analyzed after amplification of the polymorphic androgen receptor (AR) gene located on the X chromosome. XCI skewness was studied in 3 ranges: 50:50–64:36 (random inactivation), 65:35–80:20 (moderately skewed) and >80:20 (highly skewed).

Results: XCI from informative females in control group was 63% (N=19) random, 27% (N=8) moderately skewed and 10% (N=3) highly skewed. Addicted women showed 57%, 23% and 20%, respectively. The distribution and frequency of XCI status in women with opioid addiction was not significantly different from control group (P=0.55).

Discussion: Our data did not approve our hypothesis of increased XCI skewness among women with opioid addiction or unbalanced (non-random) expression of genes associated with X chromosome in female opioid addicted subjects.

1. Introduction

O pioid dependence is defined as compulsive seeking and taking of opioid drugs such as morphine and heroin and the occurrence of withdrawal syndrome when drug taking is stopped (Wise & Koob, 2014). Although many environmental elements such as stress and social factors are likely to affect drug addiction, genetic variations may also heavily influence the phenomenon (Hiroi & Agatsuma, 2005). In a study by Tsuang et al. (Tsuang et al., 1998), it has been reported that overall heritability for opioid addiction is 54% which could be bifurcated into a section accounting for sole genetic variance (38%) and another part for common liability (16%) with other substances. On the other hand, studies concerning female opioid addicts have shown that the role of genetic factors in opioid use liability in females is less significant than males (Karkowski, Prescott, & Kendler, 2000). Furthermore, a profile of a heroin-addiction epidemic reported that males form 74 percent of the addicted subjects (DuPont, 1971). However, it has been shown that craving for opioids is considerably higher among women. In addition, drug, family, medical, and psychiatric Addiction Se-

verity Index composite scores are higher in women than men (Back et al., 2011).

The most established genetic difference between males and females is due to their sex chromosomes (XX in women vs XY in men) and this factor may be involved in sex differences observed in drug addiction (Strauch & Baur, 2005). Thus, it seems reasonable to study sex chromosomes and their genetic content in disorders which their prevalence differs between two genders.

A healthy human female have two X chromosomes which one of them in each somatic cell becomes inactivated very early in embryonic development. This phenomenon which is known as X chromosome inactivation (XCI), leads to gene dosage equivalence between females and males who have only one X chromosome (Pollex & Heard, 2012). The XCI is generally a random process and the silenced X chromosome in each cell remains inactivated in all subsequent daughter cells. Thus, each tissue of an adult female is a mosaic of cells that express either the maternal or the paternal X chromosome and the average of XCI ratios are ∼50:50. However, studies have shown that XCI can be occasionally skewed or nonrandom (Amos-Landgraf et al., 2006; Busque et al., 2009; Chagnon et al., 2005).

The skewness of XCI is defined as preferential inactivation of one of the chromosomes in more than 75% of cells (Chabchoub et al., 2009; Renault et al., 2013; Talebizadeh, Bittel, Veatch, Kibiryeva, & Butler, 2005). Skewed XCI may occur as a result of an early disturbance in the process of embryo development leading to cell death followed by proliferation of small remained population of cells (Butler et al., 2007). It has been reported that XCI skewness may be involved in higher rate and manifestation of X-linked diseases in females such as X-linked mental retardation (Plenge, Stevenson, Lubs, Schwartz, & Willard, 2002), adrenoleukodystrophy (Maier et al., 2002) and Rett syndrome (Amir et al., 2000).

Furthermore, skewness of X chromosome has been studied in some multifactorial phenomenon like aging, cancer and alcoholism (Manzardo et al., 2012; Orstavik, 2006). As skewness of XCI results in the overexpression of X-linked alleles associated with one of the X chromosomes, it may lead to greater susceptibility to the development of some male-predominant disorders such as autism in affected females (Muhle, Trentacoste, & Rapin, 2004; Talebizadeh et al., 2005). Thus, we hypothesize that X chromosome inactivation may lead to increased expression of X-linked gene(s) that are involved in opioid addiction in women. To test the hypothesis, we have studied the XCI status in female subjects with chronic opioid addiction and compared them with a group of healthy women with no history of addiction.

2. Methods

2.1. Subjects

2 groups of adult females were included in the study: the first one was composed of 30 opioid addicted subjects recruited from Chitgar Campus in Tehran whose opioid addiction was confirmed according to the criteria mentioned in the Diagnostic and Statistical Manual of Mental Disorders, version 4 (DSM IV) (American Psychiatric Association, 2000). The subjects of second group were 30 healthy individuals with no history of addiction. The mean age of addicted group (± SD) at the time of sample preparation was 25.2±5.6 years.

This number was 27.4±6.3 years for control subjects. In the addicted group, dependence on other drugs of abuse such as amphetamine, alcohol, cocaine and etc was considered as an exclusion criterion. The mean duration of opioid dependence (± SD) in addicts was 3.5±2.3 years. All subjects participated voluntarily and signed a written informed consent. 3 ml peripheral blood was drawn from each subject and anticoagulated with EDTA. DNA was extracted from blood samples using QIAamp DNA Blood Kit (Qiagen). Protocols were approved by the Ethics Committee of Tehran University of Medical Sciences.

2.1.1. Determination of X chromosome inactivation

Several studies have used androgen receptor (AR) gene on the X chromosome to evaluate XCI skewness (Bittel et al., 2008; Talebizadeh et al., 2005). This gene contains a highly polymorphic region which is located at Xq13 and composed of trinucleotide repeats of CAG (Manzardo et al., 2012). There are two CpG sites near the trinucleotide repeat that are normally methylated on one of the X chromosomes in females and thus correlate with silencing. 200 ng of each DNA sample was digested with a methylation specific restriction enzyme (HpaII) as described previously (Allen et al., 1992).

The enzyme favorably degrades un-methylated (activated) over methylated (inactivated) DNA. Furthermore, each DNA sample was also digested with another enzyme (RsaI) that does not cut within the AR amplicon (here is named as "undigested"). Both digested and undigested DNA samples were used as templates for PCR amplification with Primer sequences previously described

(Giovannucci et al., 1997). The forward primer was fluorescently labeled with 6-FAM. PCR products were analyzed on an ABI 3730 sequencer (Applied Biosystems, Foster City, CA). The XCI was calculated as the ratio of the height of the shorter peak to the sum of the two peaks. For each subject, obtained data of the digested DNA were normalized with those of the undigested DNA. Thus the final formula for calculation of the XCI ratio of the shorter allele was as the following:

XCI ratio=(A/C)/ (A/C) + (B/D), where A is peak height of shorter allele (digested DNA), B is peak height of second allele (digested DNA), C is peak height of shorter allele (undigested DNA) and D is peak height of second

allele (undigested DNA), as previously described (Manzardo et al., 2012).

2.2. Statistical Analysis

According to the XCI values, subjects were divided into 3 groups: first group were subjects whose XCI ratios were 50:50 to 64:36 (random inactivation); second group were those with XCI ratios from 65:35 to 80:20 (moderately skewed inactivation) and the third group were women whose XCI ratios were >80:20 (highly skewed inactivation). Frequency of the above mentioned categories (random, moderate and highly skewed XCI) were calculated for both opioid addicted and control females. In order to compare the XCI state between two main study groups,
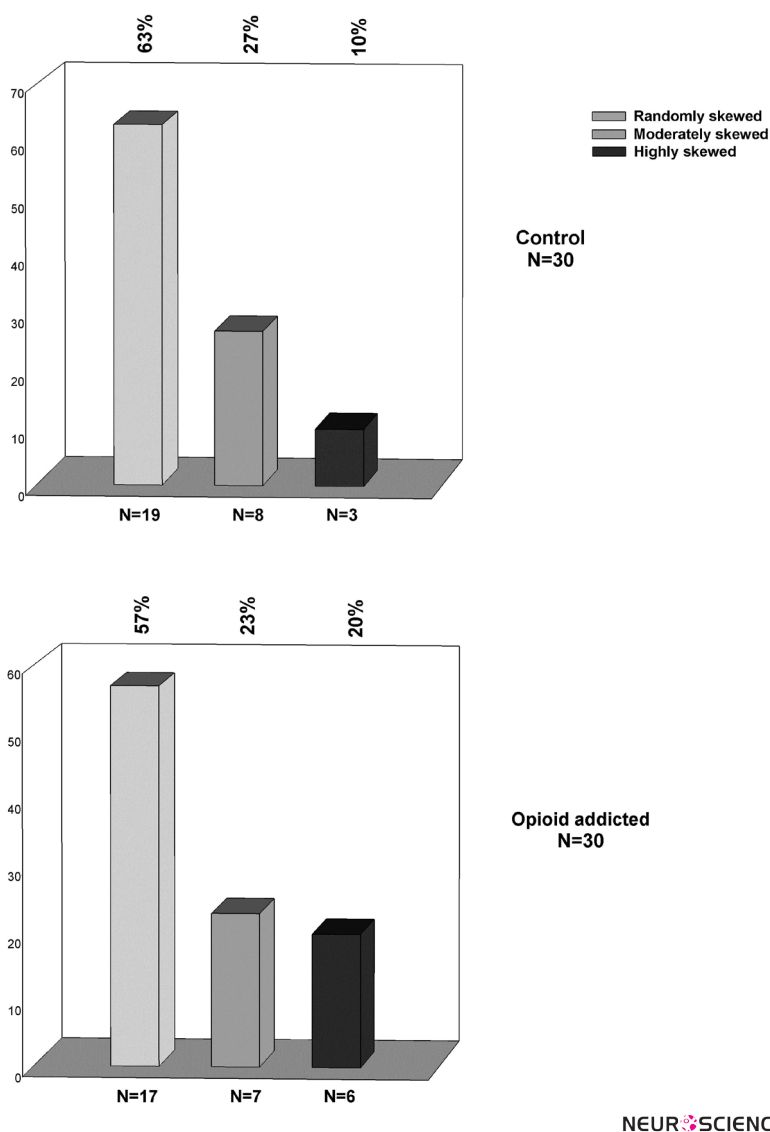
Figure 1. Distribution of patterns of XCI in 30 opioid addicted and 30 control females. XCI ratio was categorized into random (50:50%–64:36%), moderately skewed (65:35%–80:20%), and highly skewed (>80:20%). Frequency of XCI ratios in women with opioid addiction was not statistically different from control group.

we used the Chi Square test with P<0.05 as the significant level.

3. Results

XCI status was evaluated via studying the X-linked androgen receptor (AR) gene. AR gene sequence bears a well-known variation in a region of exon 1 which is considerably polymorphic and contains variable numbers of CAG repeats. This polymorphism makes this gene a valuable tool for identification of X chromosomes with maternal and paternal origin in about 90% of subjects. Thus, we have some "non-informative" cases in the normal population that are homozygous at this AR locus. We studied XCI status in 30 opioid addicted women and 30 control women with allelic heterogeneity of the AR gene.

Figure 1 represents the XCI status in females with and without opioid addiction. XCI in control group was 63% (N=19) random, 27% (N=8) moderately skewed and 10% (N=3) highly skewed. XCI of addicted women showed random pattern in 57% (N=17) of subjects, moderately skewness in 23% (N=7), and highly skewness in 20% (N=6). The distribution and frequency of XCI status in women with opioid addiction was not significantly different from control group (P-value=0.55).

4. Discussion

XCI is a complex phenomenon which is commonly random in somatic cells, with the paternal and maternal X chromosomes having an equal chance of inactivation. Random XCI in female embryos leads to equal expression of transcripts from X chromosomes with maternal and paternal origin (Wang, Yu, & Shete, 2014). However, sometimes one X chromosome may be inactivated more often than the other one. This may occur by chance or because of genetic variations on the chromosome which may make one X chromosome more prone for inactivation. Besides, if paternal or maternal X chromosome has a mutant allele whose expression leads to cell death or grown inhibition, cells inactivating the mutant allele will survive and will be selected more than cells that inactivate the normal allele.

The result would be a skewed pattern when investigating XCI in the organism or the selected tissues (Amos-Landgraf et al., 2006). Thus, the XCI ratios of females may vary from 0:100 (highly skewed), where one of the paternal or maternal X chromosomes is active in all cells, to a 50:50 ratio, where half of the cells inactivate maternal and the other half inactivate paternal X chromosome (Wang, Yu, & Shete, 2014). While skewness of XCI is a

rare phenomenon in normal females, it is rather common in situations of X-autosome translocations and in carriers of X-linked mutations. For example, skewed XCI has been reported in X-linked mental retardation female carriers (Plenge et al., 2002), females with autism (Talebizadeh et al., 2005), familial cases of Rett syndrome (Villard et al., 2001), Female carriers of X-linked adrenoleukodystrophy (Maier et al., 2002), female carriers of dyskeratosis congenita (Devriendt et al., 1997) and females with frequent spontaneous abortions (Sangha, Stephenson, Brown, & Robinson, 1999). In normal female population, highly skewness of >80:20 has been observed in about 10% of subjects (Plenge et al., 2002; Talebizadeh et al., 2005), which compares well with female controls of our study.

The number of subjects in our study was 30 controls and 30 opioid addicts which was higher than or similar to many previous researches that have investigated the XCI status in two groups of affected and control females (e.g. Bajic et al., 2015; Maier et al., 2002; Orstavik, Orstavik, Halse, & Knudtzon, 1996; Talebizadeh, Bittel, Veatch, Kibiryeva, & Butler, 2005).

Although the percentage of highly skewness was doubled in opioid addicted females in comparison to control group, our study did not show a statistical difference in the percentage or frequency of random, moderately skewed or highly skewed XCI in blood samples taken from females with opioid addiction compared to control volunteers. This result is not in agreement with the hypothesis of our study since previous researches have shown that addictive drugs may impair cell growth and division (Eisch & Mandyam, 2004; Zagon, Verderame, & McLaughlin, 2002) and thereby affect mainly dividing cells like stem and progenitor cells (Molofsky et al., 2006; Park, Morrison, & Clarke, 2004). Furthermore, the potential of these drugs to induce apoptosis (Mao, Sung, Ji, & Lim, 2002; Reece, 2007) could also add to their cell inhibition effect. These effects have specially been described for opioid drugs which are able to suppress hematopoiesis in the bone marrow and decrease the number of multi-potential progenitor cells in addition to apoptosis induction (McCarthy, Wetzel, Sliker, Eisenstein, & Rogers, 2001).

According to these data, our assumption was that XCI skewness in females with opioid addiction should be significantly higher than the control group which was not confirmed with our findings. A reason that we propose for inconsistency between our hypothesis and obtained data is based on the fact that some genes (at least 29 ones) on the X chromosome escape the inactivation process

(Helena Mangs & Morris, 2007; Ross et al., 2005). Thus, one could assume that the effect of these escaped genes may have counteracted the effect of opioids on the organism. Evaluation of this hypothesis needs further studies in the future. Although we have used blood samples for our study, researchers have found that the pattern of XCI in blood is highly correlated with that found in brain tissue (Bittel et al., 2008). Furthermore, age is an involved factor in XCI ratio because as a female subject becomes older, she gradually loses her body's hematopoietic stem cell pools and thus the ratio of XCI skewness increases in the subject (Amos-Landgraf et al., 2006). However, as the age was not significantly different in our two groups of study (data not shown), this factor may not be involved in our observed data.

Our data is in agreement with a previous study which did not find a statistical difference in XCI skewness among women with alcoholism in comparison to control group (Manzardo et al., 2012). Another possible explanation for the results obtained in our study and also alcoholic women's study may be based on a recent research reporting that the methylation of AR locus does not always correlate with XCI state (Swierczek et al., 2012). Although several studies have used this locus to evaluate XCI pattern, the statement of the mentioned research is a considerable note when discussing the data obtained from XCI studies. Furthermore, it should be noted that in the optimal state, methylation analysis is an indirect evaluation of XCI status and should be interpreted with caution.

Our study finds no evidence that skewness of XCI which may lead to over expression of X-linked genes is a risk factor for opioid addiction. Furthermore, our data propose that the effect of chronic opioid abuse on hematopoietic stem cell pools is not as much significant to cause skewed XCI in blood cells measured with our approaches when compared to controls.

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