Chromosome studies in male patients suffering from infertility

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ABSTRACT

Objective: The goal was to estimate the contribution of chromosome anomalies in the Iraqi infertile males.

Methods: Sixty-four male patients were included in the present study. Blood culture and chromosomal harvesting were conducted according to standard methods.

Results: The percentage of normal karyotype was 87.5%. The number of abnormal karyotypes constitute about 12.5%. In our azoospermic patients, about 11% of patients stated to have abnormal karyotype comparing to 15% in oligospermic patients. On the other hand, sex chromosomal anomalies were detected in 4 patients with azoospermia. No autosomal anomalies were found in this group. Meanwhile, 3 patients with sex chromosomal anomalies were recorded in oligospermic patients. The unique autosomal anomaly was detected in one oligospermic patient.

Conclusions: Karyotyping of subfertile males will still be important not only from a diagnostic viewpoint, but even more importantly, in order to gain a better understanding of gametogenic impairment, which is associated with chromosomal abnormalities. Moreover, the value of cytogenetic screening is emphasized since this group of chromosomally abnormal patients can be excluded from conventional treatment.

Keywords: Oligospermia, azoospermia, chromosome abnormalities, infertility.

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Chromosomal aberrations play a major role in male infertility. It is estimated as being between 2% and 20%.[1-4] It is some five times higher in the infertile men than in the general population. There are two main ways by which chromosome disorders can influence infertility in men. Firstly, they can disturb the development of the testis leading to a serious impairment of its normal function. Such disorders are usually caused by abnormalities of sex chromosomes.[5] Secondly, autosomal anomalies may be resulting in disruption of the normal process of cell division that occurs during the development of the gametes. With regards to their clinical expression, two major categories of chromosomal abnormalities must be differentiated. The first is due to the aneuploidy in the autosomes and induces overt pathologic abnormalities. The other, which has more discrete consequences on the carrier himself, is due to the sex chromosomal aneuploidy or balanced structural rearrangement. Indeed, the majority of anomalies, which have been observed among sterile males, attending infertility clinics, were of the second category.[6] In view of that, we have achieved a chromosomal study on a group of subfertile Iraqi subjects. The goal was to estimate the contribution of chromosomal anomalies in the Iraqi infertile males.

Methods. ***Culture condition and chromosome preparation.*** Peripheral blood lymphocytes from 64 male patients who were suffering from oligospermia

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and azoospermia were included in the present investigation. Chromosome preparations were made according to the standard methods,[7] including incubation for 72 hours in RPMI-1640. Culture media supplemented with fetal calf serum, glutamine and phytohematoagglutinin. Colchicine was added for a further two hours at a final concentration of 0.004% (w/v). The cells were then exposed to hypotonic shock and fixation in methanol: glacial acetic acid (3:1). Finally, the cell suspension was dropped to cold wet slides from a height of 30 cm. The slides were dried in a stream of cold air.

Giemsa stain technique. Air dried slides were placed horizontally on staining racks and flooded with 10% (v/v) Giemsa in phosphate buffer saline for 10 minutes, followed by a brief washing in running tap water. The slides were then mounted in depex after air-drying.

Results. Out of a total of 64 infertile men only 8 patients revealed an obvious chromosomal aberration, which accounted to 12.5%. Table 1 shows that out of 37 azoospermia, 4 patients had abnormal karyotypes, which accounted to 11%. Only 4 oligospermic patients (15%) showed an obvious chromosomal aberration out of a total of 27 patients with low sperm count who were subjected to the present investigation. Table 2 summarizes the type of chromosomal abnormalities, which have been detected in 4 azoospermic patients. It is clear, that the sex chromosomal abnormalities were the sole causes. No autosomal abnormalities were associated with azoospermia in our results. Autosomal abnormality was found in only one oligospermic patient who represents a percentage of 4% (Table 3). Three other oligospermic patients were found to have sex chromosome anomalies constitutes 11%. The chromosomal constitutions of 8 infertile men who showed an obvious chromosomal aberration are grouped in Table 4. Karyotype investigation revealed that 4 azoospermia patients showed 47, XXY male mitotic karyotype, which is associated with Klinefelter's syndrome. No other abnormalities were noticed. Three oligospermia patients revealed a mosaic 46, XY/47, XXY male mitotic karyotype, which is associated with Klinefelter's syndrome although the mosaic ratio seems to be different among them. (Table 4). The majority of these patients did not have a typical Klinefelter syndrome signs (e.g. small testes, Gynecomastia). One azoospermic patient showed 46, XY/45, XY,t(13;14) male (Figure 1) with mosaic ratio of 70:30. Fifty-six infertile men (azoospermia and oligospermia) revealed 46, XY males with no obvious chromosomal aberration seen.

Table 1 - Chromosome studies of infertile men - azoospermia and Oligospermia.

Type of infertility	No. of patients analyzed	No. of normal karyotype (%)	No. of abnormal karyotype (%)
Azoospermia	37	33 (89)	4 (11)
Oligospermia	27	23 (85)	4 (15)
Total	64	56 (87.5)	8 (12.5)

Table 2 - Type of chromosome abnormalities among azoospermia.

Type of chromosome abnormalities	Number of patients	Anomaly detected (Total=37) (%)
Sex-chromosomal abnormalites	4	11
Autosomal abnormalities	0	0
Total	4	11

Table 3 - Type of chromosome abnormalities in 4 oligospermia.

Type of chromosome abnormalities	Number of patients	Anomaly detected (Total=27) (%)
Sex-chromosomal abnormalites	3	11
Autosomal	1	4
Total	4	15

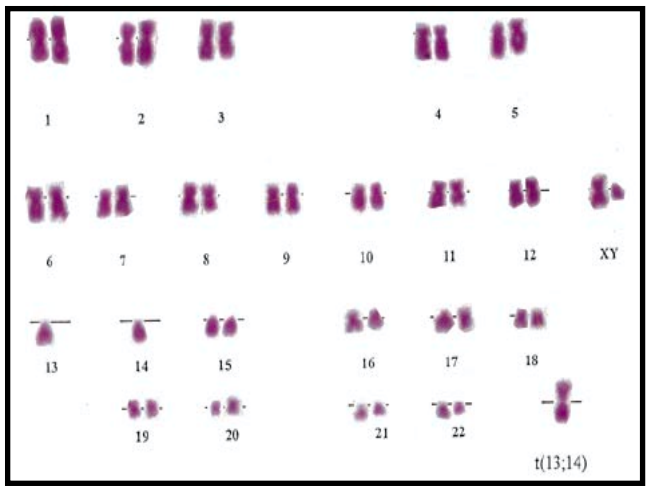

Figure 1- Shows Giemsa stain karyotype of oligospermic patient with 13/14 translocation.

Table 4 - Chromosomal constitution of 8 patients with chromosomal abnomalities.

Individual No.	Age	SFA*	Chromosome constitution	Mosaicis m ratio	Autosome/sex abnormalities ratio
Y1	42	Azoospermia**	47, XXY	Non	
Y2	30	Azoospermia	47, XXY	Non	
Y3	34	Azoospermia	47, XXY	Non	
Y4	37	Azoospermia	47, XXY	Non	
Y5	40	Oligospermia***	46, XY/47, XXY	50:50	1:7
Y6	51	Oligospermia	46, XY/ 47, XXY	30:70	
Y7	34	Oligospermia	46, XY/47, XXY	45:55	
Y8	45	Oligospermia	46, XY/45, XY, t(13:14)	70:30	

*Seminal fluid analysis, **Azoospermia (sperm count is equal to zero), ***Oligospermia (sperm count is less than 10 millions/ml), SFA-subfertile abnomalities

The present investigation showed an incidence of 12.5% (Table 1) which tends to be in the same range of that reported elsewhere.[3,4] Furthermore, Table 1 shows that about 11 of azoospermic patients who were subjected to this investigation were chromosomaly abnormal which was in coincidence with that reported by Chandley,[2] who reported a percentage of 15%, and that of Retief et al[10] who reported a percentage of 14%. However, among the oligospermia, only 15% showed abnormal karyotype in our study. This percentage seems to be higher than 2% that is reported elsewhere.[2]

Discussion. Since it became established that specific sex chromosomal aneuploids are associated with male infertility,[8] groups of men attending infertility clinics were being screened by cytogeneticists to determine the frequency of abnormalities in selected or unselected populations. The results of Kjesseler[9] reported an overall incidence of 7%, whereas, Chandley[2] reported an incidence of 2% in his cytogenetic screening of 2,372 men presenting with infertility. On comparing, other investigators stated a high incidence of 13%.[3] Also, a more recent study registered an incidence of chromosomal abnormality to 13%.[4] Obviously, the present study dealt with the infertile patients who were severely oligospermic. Previous studies have proposed a positive relationship between chromosomal abnormality and sperm count. Accordingly, the above statement may explain to some extent this higher frequency although other reasons (e.g. race, environmental factor) can not be excluded. On the other hand, the ratio of autosomal to sex chromosomal abnormalities is estimated in the present investigation to be 1:7. The Robertsonian's translocation in the D group is the unique abnormalities observed and occurring in a frequency of 4%. Again, these results are higher than other studies. In the survey of Retief et al,[10] the ratio of autosomal to sex chromosomal abnormalities was 1: 2.5 and the frequency of Robertsonian's translocation was reported to be 1%. The results consolidate our previous explanation that sperm count, race, environmental factor may play a vital role in the frequency of chromosomal abnormality among the infertile patients in Iraq. These factors may act together each with small, but additive effects that lead finally to infertility. Sex chromosomal abnormalities seem to be the sole cause of azoospermia. No autosomal abnormalities that are associated with azoospermia were observed. This observation is in completeagreement with that reported by others.[10] In oligospermia, sex chromosomal abnormalities were detected. Furthermore, autosomal abnormalities were found among the oligospermia. Other studies showed an autosomal abnormality among the oligospermia.[10] The above two results are almost similar although our result was slightly higher probably owing to previously mentioned reasons. It is highly apparent from that Klinefelter's syndrome is the most frequent sex chromosomal abnormality. The classic form of Klinefelter's syndrome revealed 47, XXY male mitotic karyotype which was associated with azoospermia, while the mosaicism form showed a 46,

XY/47, XXY male mitotic karyotype. It must be mentioned here, that the mosaicism ratio was not the same in all of the cases that were detected. The difference in the mosaicism ratio affects the phenotypic traits of the patients. Indeed, all the mosaic forms of Klinefelter's syndrome were associated with oligospermia rather than azoospermia as might be expected. Thus, one might be believed that more 46, XY chromosomal constitution in the mosaic complement might decrease sterility and cover much of the clinical features of the patients. Oligospermia has seldom been reported in subjects affected by Klinefelter's syndrome, and those associated with various degrees of tubular alteration, arrest of maturation process at primary spermatocyte or spermatid level and the presence of spermatogenesis only in rare seminiferous tubules.[12,13] It has been suggested in Klinefelter's syndrome that the spermatogenesis was related to the presence of normal 46, XY germ cells,[14] and the different degrees of testicular alteration depend on the proportion of normal 46, XY tubular cells, including germ cells and cells surrounding them.[15] One patient with DqDq translocation was identified which was associated with oligospermia. Robertsonian's translocation was reported to reduce male fertility.[16] The D/D translocation is the most common type.[17] Furthermore, varying degrees of spermatogenesis impairment have been reported among carriers of both DqDq and DqGq translocations and the spermatogenic arrest was observed in spermatocyte or spermatid.[5] No D/G or G/G translocations were observed in the present investigation. Although it's difficult to determine whether the rare G/G translocation has a greater effect upon male reproduction fitness as has been suggested by others.[18] Several studies have reported that subfertility in translocation carriers can be brought about in two ways.[19] Firstly, it can result from the production of genetically unbalanced gametes, which lead to spontaneous abortion of unbalanced zygotes. Secondly, subfertility can be the consequence of the spermatogenic disturbances, which result in oligospermia and azoospermia. To this end, it seems that karyotyping of subfertile males will is still important not only from a diagnostic viewpoint, but also even more importantly in order that we gain a better understanding of gametogenic impairment, which associated with chromosomal abnormalities. Moreover, the value of cytogenetic screening is emphasizing since this group of chromosomally abnormal patients can then be excluded from conventional treatment.

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