The role of Y chromosome C– band size polymorphism in male infertility with a reference to their effect on the total length of the chromosome

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ABSTRACT

Objective: To assess the contribution of chromosome on sperm production.

Methods: During a 2 year period from October 1999 to December 2001 a total of 200 male patients were included in the present study. The study was carried out at Kufa Medical College, Kufa, Iraq. Blood culture, chromosomal harvesting, Giemsa stain, C-band technique and C-band size calculation have been conducted according to the standard methods.

Results: No statistical differences have been recorded in the C-band size distribution level among the infertile groups compared to normal control. The functional relationship between C-band, euchromatin and the total length of the Y chromosome showed a strong correlation coefficient among the infertile groups (oligospermia, azoospermia) as well as the normal control.

Conclusion: In our qualitative study the C-band size of the Y chromosome has no effect on spermatogenesis. The decrease in the size of Y chromosome is due to the decrease in both the heterochromatin and euchromatin regions.

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Human Y chromosome studies started at the same time when chromosome analysis was started in the beginning of 1950.[1] It is one of the smallest chromosome with an estimated average size of 60 mbp.[2] It is subjected to great inter-individual variability due to size variation of the heterochromatin region,[3] which in most cases constitute less than 50% of the chromosome. It contains a high number of repetitive elements[4] region that are homologous to the X chromosome[5] and few functional genes are generally restricted to the euchromatic region on the short and proximal long arm,[6] including the determinant factor for the male sex.[3] Indeed, Tiopolo and Zuffard[7] were the first to propose that Y chromosome deletions interfere the male fertility. Those authors observed terminally deleted Y chromosome in the karyotype of 6 sterile males with azoospermia. The deletion includes the large heterochromatin block in the long Y arm (Yq12) and an undefined amount of adjacent euchromatin part (Yq11). Few years later, the presence of azoospermia factor (AZF) in Yq11 was confirmed by numerous studies of cytogenetic level[8] and at molecular level.[9-11] On the other hand, further extensive cytogenetic studies that dealt with male infertility have shown that there was an obvious role

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for many chromosomal factors in male infertility. Those factors include Y chromosome polymorphism,[12] dicentric Y chromosome[13] pericentric inverted Y chromosome,[14] pericentric Y chromosome with minute deletion[15] and a ring chromosome.[16] The present study was designed to assess the possible role of C-band polymorphism of the Y chromosome in male infertility. This approach was born in mind as the gene responsible for spermatogenesis is located near the heterochromatic region of the Y chromosome.[17] Thus, any change in the size of the heterochromatic region may have direct or indirect effect on the gene responsible for the sperm production.

Methods. During 2 years period starting from October 1999 to December 2001, a total of 200 infertile male patients and 100 normal fertile men were subjected to the present investigation. All our patients were assessed by consultant urologists and by clinical laboratory test. Culture media preparation, chromosome cytology, Giemsa stain, C-banding technique and C-band size calculation have been published elsewhere.[18-22]

C-band size polymorphism calculation. The size of C-band is classified into 5 levels (1-5) according to Patil and Lubs suggestion.[23] The parameters, which have been used, depend on the size of the short arm of chromosome number 16. The total length of C-banded Y chromosome was calculated directly from photograph by measuring the length of the positive C-band (presented in micrometers) and the length of the negative C-band region. Accordingly, for human Y chromosome C/F, E/F and Y/F ratios were calculated whereas C is the average length band size (ςm), E is the average length of C-band negative region (ςm), Y is the average of the total length of Y chromosome, F is the average length of group F chromosome.

Statistical analysis. The results of the investigation were statistically evaluated using the chi-square test (Yatte correction factor). Correlation coefficient was also used and the hypothesis was tested by t-test study.

Results. Eighteen patients out of 80 were suffering from oligospermia with level one Y chromosome C-band size that accounted for 22.5%. Again, 20 azoospermia patients out of 120 showed level one C-band size that accounted for 16.7%. As far as level 2 C-band size is concerned, 28 patients with oligospermia showed level 2 C-band size of 35%, 57 of the azoospermia showed level 2 C-band size of 47.5% and 35 normal control group showed level 2 C-band size of 35%. Furthermore, 34 oligospermic, 43 azoospermic and 51 normal fertile men have shown an obvious level 3 C-band size, which reached 47.5%, 35% and 51% (**Table 1**). No other C-band level has been recorded in our results. No statistical differences could be detected in the distribution of C-band size of oligospermia, azoospermia as compared to normal fertile group.

C-band euchromatin and Y chromosome length. The mean number of Y/F, C/F and E/F ratios among the azoospermia, oligospermia and normal group were indicated in **Table 2**. A highly significant correlation has been found between C/F versus Y/F among the azoospermia patients (t=20.2, $p<0.05$), the normal control group showed likewise a highly significant correlation coefficients (t =24.24, $p<0.05$). When the E/F versus Y/F values were correlated, the result showed a significant correlation among the azoospermia, oligospermia and control groups (t=13.1, $p<0.05$) and (t=12.4, $p<0.05$) and (t=40.3, $p<0.05$).

Finally, C/F values were correlated to E/F for all the groups, which were subjected to the present study, the result of correlation coefficient showed no significant correlation was found with regard to C/F versus E/F values (t=0.75, $p>0.05$), (t=0.717, $p>0.05$) and (t=0.59, $p>0.05$).

Discussion. It is well established that the Y chromosome in male, as in most organisms, lacks functional genes except for those involved in fertilization and male fertility.[24] In fact, after the

Table 1 - Distribution of C-bands Y chromosome according to their size.

Level	Y chromosome heterochromatin		
	Group	N of cells measured	C-band size n (%)
1	Oligospermia	240	18 (22.5)
1	Azoospermia	360	20 (16.7)
1	Control	300	10 (10)*
2	Oligospermia		28 (35)
2	Azoospermia		57 (47.5)
2	Control		35 (35)
3	Oligospermia		34 (47.5)
3	Azoospermia		43 (35)
3	Control		51 (51)*
4	Oligospermia		-
4	Azoospermia		-
4	Control		4
5	Oligospermia		-
5	Azoospermia		-
5	Control		-

In level 1 x^2 for oligospermia and control was 0.9, x^2 for azoospermia and control was 0.65 and the degrees of freedom was 1,
In level 2 x^2 for oligospermia and control was 1.4, x^2 for azoospermia and control was 1.9 and the degrees of freedom was 1,
In level 3 x^2 for oligospermia and control was 1.7, x^2 for azoospermia and control was 1.87 and the degrees of freedom was 1.
All of them were non significant ($p>0.05$)

Table 2 - Statistical comparison between oligospermia, azoospermia patients and normal fertile group.

Component	Oligospermia	Azoospermia	Control
A-mean			
Y/ F	1.1 ± 0.06	1.05 ± 0.02	1 ± 0.18
C/F	0.6 ± 0.02	0.6 ± 0.02	0.5 ± 0.2
E/F	0.5 ± 0.05	0.68 ± 0.022	0.46 ± 0.06
B-correlation (r)			
C/F versus Y/F	(0.95)*	(0.99)*	(0.94)*
E/F versus Y/F	(0.4)*	(0.6)*	(0.81)*
C/F versus E/F	(0.32)	(0.08)	(0.38)

*significant $p<0.01$. Y - average length of Y chromosomes, F - average length of F group chromosomes, C - average length of C-band regions, E - average length of euchromatin region

first description of large microscopically visible deletion of the Y chromosome in metaphase spreads of infertile male,[7] the effect of several discrete tracts on the Y chromosome that contains genes probably involved in male genetic maturation have been studied extensively.[25] Today, it is well known that the azoospermia factor (AZF), which contains at least 2 well-characterized genes families recognition binding motion (RBM) and deleted azoospermia (DAZ), located on the distal part of the long arm of the Y chromosome. At present, it seems that deletion of the RBM genes in the Yq11 (interval 6)[26] is sufficient to impair spermatogenesis. It seems likely that all genes responsible for sperm production are located on the euchromatin region of the Y chromosome long arm. Thus, C-band size, which is usually located on the same arm, may have some sort of effect on sperm count. According to our results, no statistical differences could be detected in the distribution of C-band size in both infertile groups as compared to the normal fertile group (**Table 1**), in other words, the size of the heterochromatic region has no direct or indirect effect on spermatogenesis since both infertile groups and the normal control showed almost the same C-band size distribution level.

On the other hand, when a functional relationship between positive C-band region, euchromatin and the total length of the Y chromosome using the variable (C/F versus Y/F, E/F versus Y/F) in both infertile and the normal infertile groups, a highly significant correlation has been noticed between C/F versus Y/F among both oligospermia and azoospermia and the normal control (**Table 2**). Again E/F versus Y/F also showed a significant correlation among the infertile groups and normal fertile group. No significant correlation was noticed when C/F values versus E/F were correlated. Our finding indicates that the length of the Y chromosome is dependent on the euchromatin region as well as the C-band region (heterochromatin) and there was a strong correlation between the C-band region and the euchromatin (namely the decrease in the size of the Y chromosome is due to the decrease of both the heterochromatin and euchromatin regions).

These results are consistent with other published studies. Furthermore, our results indicate that there is no effect on the size of C-band region on sperm production either directly or indirectly, though further extensive study is highly recommended before any conclusion could be reached.

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