The significance of autosomal C-band size polymorphism in male infertility

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ABSTRACT

Objective: The aim of this study is to assess the possible role of autosomal C-band size polymorphism in male infertility.

Methods: Two-hundred male patients with clinical diagnosis of infertility and 100 normal controls were included in the present investigation. All patients were assessed by Urologist Consultant at the Department of Pathology and Forensic Medicine, Kufa University, Kufa, Iraq, during a 2-year-period, October 1999 to October 2001. C-band evaluation was based on both quantitative and qualitative methods. Blood culture, chromosome harvesting, and C-band technique were carried out according to standard methods.

Results: 1. C-band quantitative study indicates a significant increase in the C- band size of chromosomes 9 and 16 among infertile groups as compared to normal fertile group ($p<0.01$). 2. C-band qualitative study indicates a significant increase in the C-band size (level 3) of chromosome number one among the infertile group as compared to normal fertile group ($p<0.01$).

Conclusion: The present findings require further extensive study to shed light on the possible correlation between C-band polymorphism and male infertility.

Saudi Med J 2002; Vol. 23 (12): 1473-1477

The constitutive heterochromatin consists mainly of highly repetitive DNA, which is largely equivalent to the C-band centromeric region of the autosomes. The polymorphism of autosomal C-band positive region which is usually located on chromosomes 1, 9 and 16 are inherited in a mendelian manner[1] and it shows a great population and evolutionary stability, though ethnic differences have been reported.[2] However, sex and age-related C-band differences of the autosomes have not been reported yet.[1,2] It is believed that the polymorphism in the C-band region of chromosome one may predispose to malignant disease, particularly in the carcinoma of the ovaries and cervix uteri.[3] Similar associations have been reported between breast cancer and C-band heteromorphism of chromosomes one, 9 and 16[4] though other investigators deny such associations, especially when a soft tissue sarcoma was used.[5] On the other hand, C-band polymorphism was thought to have some deleterious effect on some patients and may induce some chromosomal aberrations.[6,7] It has been reported that in 15% out of 44 patients who were carrier of $1qh^+$ and $9qh^+$, C-band variants had shown to have an obvious association with chromosomal aberrations[8] and repeated spontaneous abortions.[9] Indeed, the present investigation is designed to evaluate the possible role of autosomal C-band size polymorphism on sperm count and spermatogenesis in general. To our knowledge, no other studies used the same parameters have been reported elsewhere.

Methods. During a 2-year-period (October 1999 to October 2001) a total of 200 male patients consisting of 120 azoospermia and 80 oligospermia

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Received 10th March 2002. Accepted for publication in final form 24th August 2002.

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together with 100 normal control were subjected to the present investigation. All our patients were assessed by Consultant Urologist and by seminal analysis test. Culture conditions and chromosome cytology that has been published previously.[10-12]

C-banding technique. C-banding technique was carried out by a modification of the method of Arrighi et al.[13] Freshly prepared slides, 1-3 days old, were placed in a freshly prepared 5% (weight/ volume) saturated aqueous solution of barium hydroxide octahydrate [Ba $(OH)_2$ $8H_2O$] for 5 minutes at 37°C. 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 60-65°C 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.

C-band size polymorphism calculation. The calculation of C-band length was preformed directly from a positive photograph using the linear measurement method that has been suggested elsewhere.[14,15] The absolute C-band length measurements are presented in micrometer and the relative length measurements as percentage. In qualitative study, the length of heterochromatin regions were classified into 5 levels (level 1, 2, 3, 4, and 5) according to the qh region of the short arm of chromosome 16 that has been suggested by others.[16] All results were statistically evaluated using Z-test and analysis of variance (ANOVA) test.

Results. ***1) C-band quantitative study.*** The ANOVA test which has been applied to evaluate the C-band absolute mean size between infertile and fertile groups of chromosomes 1, 9, and 16, show an obvious differences between them ($F=43.33$, $P<0.05$) (**Table 1 and Figure 1**). However, when each individual chromosome is compared with each other using the Z-test analysis, no significant differences has been recorded in the C-band absolute size of chromosome one of both oligospermia and azoospermia as compared to chromosome one of normal fertile group ($P>0.01$). However, a significant increase in the C-band absolute size of chromosomes 9 and 16 of both infertile groups has been noticed as compared to the same chromosome among normal fertile group ($P<0.01$). With regard to the C-band relative length, the ANOVA test showed no overall differences between both infertile and fertile groups. Indeed, when the means of each individual chromosomes were compared separately, the Z-test revealed a significant increase in C-band relative length of chromosome 9 of oligospermia patients as compared to chromosome 9 of normal men ($p<0.01$). No significant differences in C-band size relative length of this chromosome among the azoospermia patients as compared to the same chromosome of the normal control ($P>0.01$). Again, the C-band relative length of chromosome 16 showed a highly significant increase in both infertile groups as compared to chromosome 16 among fertile group ($P<0.01$) (**Table 2**). As far as, chromosome one is concern, no significant increase or decrease in C-band relative length could be noticed between the infertile and fertile groups ($P>0.01$).

2) C-band qualitative study. In our C-band qualitative study, the C-band is classified into 5 levels according to their size (level 1, 2, 3, 4, and 5). It is clear from **Table 3** that there was a significant increase in level 3 C-band (large size) of chromosome one among both the oligospermia and azoospermia as compared to the same chromosome

Table 1 - Absolute lengths of C-band on chromosome 1, 9 and 16 in patients (oligospermic, azoospermic) and the control group.

Groups	Chromosomes					
	1		9		16	
	Larger (m ± SD)	Smaller (m ± SD)	Larger (m ± SD)	Smaller (m ± SD)	Larger (m ± SD)	Smaller (m ± SD)
Azoospermic n = 120	3.2 ± 0.5	2.1 ± 0.86	2.1 ± 0.74	1.7 ± 0.74	1.78 ± 0.29	1.58 ± 0.36
	2.7 ± 0.68		1.9 ± 0.6*		1.68 ± 0.6*	
Oligospermic n = 80	2.99 ± 0.69	2.76 ± 0.65	2.4 ± 0.37	2.23 ± 0.43	1.81 ± 0.27	1.4 ± 0.23
	2.89 ± 0.67		2.33 ± 0.4*		1.61 ± 0.25*	
Control n = 100	2.74 ± 0.45	2.64 ± 0.4	1.99 ± 0.28	1.59 ± 0.26	1.48 ± 0.42	1.08 ± 0.29
	2.69 ± 0.425		1.79 ± 0.27		1.28 ± 0.355	

*Z test = significant $P<0.01$. Comparison: azoospermic, oligospermic, control are significant differences F - test RCBD analysis of variance, m ± SD - mean ± standard deviation

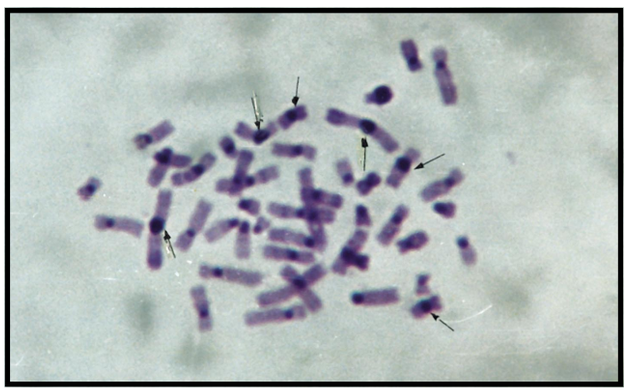

Figure 1 - C-banded metaphase spread of infertile male patients with normal chromosome constitution 46XY, note the centromeric heterochromatin (C-band) on chromosome 1, 9 and 16 (see arrow).

Table 2 - Relative lengths of C-band on chromosomes 1, 9 and 16 on patients (oligospermic, azoospermic) and the control group.

Groups	**Chromosomes**					
	1		**9**		**16**	
	Larger (m ± SD)	**Smaller (m ± SD)**	**Larger (m ± SD)**	**Smaller (m ± SD)**	**Larger (m ± SD)**	**Smaller (m ± SD)**
Azoospermic n = 120	23.1 ± 3.84 22.37 ± 3.74	21.5 ± 3.596	19.8 ± 5.56 18.81 ± 4.32	17.8 ± 3.08	18.5 ± 2.12 17.76 ± 2.41	16.46 ± 2.7
Oligospermic n = 80	22.23 ± 3.12 21.67 ± 3.49	21.11 ± 3.86	20.43 ± 4.09 19.57 ± 4.415*	18.71 ± 4.74	17.1 ± 3.29 16.1 ± 3.51*	15.1 ± 3.73
Control n = 100	20.98 ± 6.06 20.3 ± 5.8	19.62 ± 5.63	17.7 ± 3.6 17.2 ± 3.87	16.7 ± 4.13	12.9 ± 3.98 12.4 ± 3.82	11.9 ± 3.66
*Z test = significant P<0.01. Comparison: azoospermic, oligospermic, control are not significant differences F - test RCBD analysis of variance P<0.05 m ± SD - mean ± standard deviation						

Table 3 - Distribution of homologous chromosomes 1, 9 and 16 according to C-band size in azoospermic, oligospermic, and control groups.

Level	**Groups**	**n of cells examined**	**Chromosome 1 n (%)**	**Chromosome 9 n (%)**	**Chromosome 16 n (%)**
1	Oligospermic	80	5 (6.2)	8 (10)	60 (75)
1	Azoospermic	120	-	22 (18.3)	104 (86)
1	Control	100	-	4 (4)	24 (24)‡
2	Oligospermic	80	40 (50)	40 (50)†	20 (25)
2	Azoospermic	120	62 (51.6)	111 (92)	60 (41)
2	Control	100	78 (78)	96 (96)	76 (76)
3	Oligospermic	80	38 (47.9)	30 (37.5)	-
3	Azoospermic	120	52 (43.3)*	8 (6.6)	-
3	Control	100	22 (22)	-	-
4	Oligospermic	80	-	-	-
4	Azoospermic	120	-	-	-
4	Control	100	-	-	-
5	Oligospermic	80	-	-	-
5	Azoospermic	120	-	-	-
5	Control	100	-	-	-
n - number, *significant differences = P<0.05, x^2 = 60.78, df = 1, level 3 to chromosome 1 for comparison to azoospermic and control. †significant difference = P<0.05. x^2 = 6.8, df = 1, level 2 to chromosome 9 for comparison to oligospermic and control. ‡significant difference = P<0.05, x^2 = 12.3, df = 1, level 1 to chromosome 16 for comparison to oligospermic and control, df - degrees of freedom					

of normal control group (X^2= 60.78, df=1, P<0.05). Indeed, the percentage of cells which showed level 3 C-band size in chromosome one reached a frequency of 47.9% in the oligospermia and 43.3% in the azoospermia compared to normal control with approximately 22%. On the other hand, no significant increase or decrease has been recorded in the small size C-band (level 1) or (level 2) in chromosome one in either the oligospermia or azoospermia compared to fertile group. Furthermore, a significant increase in the C-band size (level 1) of chromosome 16 has been noticed among both infertile groups (X^2= 6.8, df=1, P<0.05) as compared to the same chromosome of normal fertile men. The percentage of cells which showed level one C-band in chromosome 16 among the oligospermia were 86%, azoospermia were 75% and the control group were 24% (**Table 3**). No other significant results could be detected.

Discussion. It is well established that the majority of infertile patients revealed (46, XY) male mitotic Karyotype with no obvious chromosomal aberration could be detected, thus, C-band studies have been conducted in an attempt to shed the light if C-band polymorphism may resolve the clue. Generally speaking, C-band polymorphism was thought to have some deleterious effect on patients who carry $1qh^+$ and $9qh^+$ C-band variants and out of 36 couples only 6% showed no obvious chromosomal aberration, yet a spontaneous abortion had occurred.[17] Although no clear picture could be reached, most investigators who dealt with C-band variants correlated the deleterious effect with larger C-band size.[1] In the present investigation, a significant increase in C-band size (absolute and relative length) of chromosomes 9 and 16 have been recorded among the oligospermia and azoospermia patients as compared to normal control group (P<0.01) (**Table 1**). No such observation was noticed with regard to C-band size of chromosome one. Furthermore, no significant differences were recorded when both infertile groups were compared with each other. If we accept the notion that the larger C-band may have a deleterious effect as has been proposed elsewhere,[18,19] the inevitable question need to be answered then, why a high frequency of larger C-band could be revealed among normal population.[20] It is believed that C-band variants may play a role in the karyotyping evolutionary stability,[21,22] thus, the C-band variability is now in equilibrium and the heteromorphism has been lost by natural selection which occurred by either the mutation or by preferential segregation. Accordingly, the large C-band size may have either direct or indirect effect, which may influence on sperm production. On the other hand, our qualitative study showed 3 C-band sizes distribution levels (1, 2 and 3). A significant increase in the frequency of C-band large size (level 3) has been noticed among both fertile groups as compared to normal fertile group (P<0.05). Again, a significant increase in the frequency of small size (level 1) C-band in both the infertile groups compared to a normal control. On the contrary, a significant decrease in the frequency of level 2 C-band size among the infertile group in comparison with the fertile group has been reported. The results gave as an obscure picture which push us to rely mainly on the quantitative study in our C-band evaluation. Indeed, the qualitative study depends chiefly on the relative measurement of the short arm of chromosome 16. This is a small size chromosome and the less affected one by colchicine treatment and cell cycle duration.

In conclusion, the significant increase in the absolute and relative C-band size of chromosomes 9 and 16 among the infertile group is consistent with those reported earlier which correlate the large C-band with some reproductive fitness as the abortion.[8] Our findings may goes under the same umbrella. Obviously, the large C-band on chromosomes 9 and 16 may have indirect effect on sperm production. It seems likely that the molecular basis of male infertility and fertility is not a linear order of genetic events, but the result of interaction of complex genetic networks that function in 3 main pathways; male germ line development, male gonad development, and male somatic development. Consequently, primary genetic switch signals should be exist for linking the different genes network and for starting them. Indeed, there is some evidence, which concluded that the switch signals are concentrated on the sex chromosomes.[23] Accordingly, multiple genes encoding for male fertility exists also on other chromosomes (autosomes). Thus, if these genes are located in or near the C-band positive region it will undoubtedly be effected. Accordingly, if the C-band size is increased it may have indirect effect on sperm production that affect the signals network in general.

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