High frequency of satellite association in metaphases of infertile male patients

Akeel A. Yasseen, MSc (UK), PhD (UK), Aida F. Aunuiz, MSc.

ABSTRACT

Objective: The present study is designed to evaluate the contribution of satellite association phenomenon on spermatogenic impairment.

Methods: During a one year period, January 1999 through to January 2000, the frequency of satellite association has been investigated in 57 patients, with the clinical diagnosis of male infertility. This study was carried out at the Middle Euphrates Center for Cancer Researches, College of Medicine, Kufa University, Kufa, Iraq. Of those 57 patients, 33 patients were diagnozed with azoospermia and 24 patients with oligospermia, the efficacy evaluation was based on the measurement of 8 satellite association parameters. Blood culture and chromosome harvesting was carried out according to our standard methods.

Results: Our experience of association behavior of acrocentric chromosome in the 57 infertile males showed statistically significant difference in infertile male classes compared to control groups. Eight studied parameters were included in the evaluation ($P<0.005$).

Conclusion: The significant increase in the satellite association is proposed to have another indirect causal factor, which influenced spermatogenesis. Furthermore, the satellite association technique may be used as a laboratory method for the evaluation of male infertility.

Keywords: Satellite, association, metaphases, male infertility, azoospermia, oligospermia.

Saudi Med J 2002; Vol. 23 (4): 427-431

Fundamentally, each chromosome has a characteristically located constriction, the centromere, which may help orient the chromosome during cell division. The centromere position distinguishes the chromosomes. A chromosome is acrocentric if the centromere pinches off a small amount of material. Acrocentric chromosomes also have blob-like ends called satellites that extend from stalk like bridge from the rest of the chromosome. In fact, the association of acrocentric chromosomes by their satellite ends is a well-documented phenomenon.[1,2] However, the acrocentric association frequency varies between the individual and the basis of the variation and is thought to be an extensive polymorphism for associating ability in each homologous class of nucleolus-organizing chromosomes.[3] It has been considered that the characteristic tendency of each chromosome to be associative, contributes to the overall pattern of spatial relationships of the entire complement.[4] So far, many evidence revealed in cases where the individual marker chromosomes can be followed, that there are indications that the associating ability of a chromosome is inherited.[5] It is also known that the frequency of the satellite association can vary in the same individuals according to different physiological conditions, such as the amount of thyroid hormones[6] as well as the different cell culture conditions.[7] Other factors such as different techniques involved in chromosome spreading and preparation can also have a marked effect on the frequency of satellite associations.[8] Indeed, particular

From the Department of Pathology and Forensic Medicine, (Yasseen), Middle Euphrates Center for Cancer Researches, (Aunuiz), College of Medicine, Kufa University, Kufa, Iraq.

Received 15th June 2001. Accepted for publication in final form 30th September 2001.

Address correspondence and reprint request to: Dr. A. Yasseen. Professor/Consultant Medical Genetics, Department of Pathology and Forensic Medicine, College of Medicine, Kufa University, PO Box 21, Kufa, Iraq. Tel. +964 (33) 346034 Fax. +964 (1) 8867170. E-mail: kufa@uruklink.net

attention has been directed towards the correlation between satellite association tendency and the incidence of anomalies involving acrocentric chromosomes.[9-11] Their aim was to specify the involvement of acrocentric associations in the occurrence of chromosomal non-disjunction and Robertsonian's translocation.[12,13] A high frequency of specific acrocentric association has been considered as a predisposing factor to meiotic and mitotic non-disjunction. In the present communication we present data on the acrocentric association among the infertile patients who were suffering from either oligospermia or azoospermia. The goal was to evaluate the contribution of the satellite association phenomenon on spermatogenesis.

Methods. ***Patient selection.*** During a one-year period, January 1999 through to January 2000, a total of 57 infertile men (33 azoospermic and 24 oligospermic patients) were subjected to the present study. The study was carried out at Middle Euphrates Center for Cancer Researches, Kufa University, Kufa, Iraq. The ages ranged from 18-55 years. A corresponding number of fertile men were included for comparison. All the patients were assessed by a Consultant Urologist and by seminal analysis test.

Culture media and chromosome harvesting. Chromosome cytology was conducted according to our standard methods.[14,15] Peripheral blood lymphocytes were cultured in RPMI culture media buffered by sodium bicarbonate. The cultures contained (50) units per ml or (100μl/ml) benzyl penicillin and (100μl/ml) streptomycin, 2% phytohematoagglutinin and 20% fetal bovine serum. Metaphase cells were accumulated by 2-hour treatment with colchicine (sigma) at a final concentration of 0.004%(w/v). Afterwards the cells were treated with 0.075M kaluim chloride (KCI) for 30 minutes and fixed in 3 changes of ice-cold methanol: glacial acetic acid (3:1) before spreading in a stream of cold air. Normal chromosome staining was carried out with 10%(v/v) Giemsa (Raymond A. Lamb) in phosphate buffer saline (pH 6.8) for 10 minutes. Satellite association was scored if the satellite ends of 2 or more acrocentric chromosomes lay within 1.2μm of each other. For all cases, we scored 25 cells per sample. To avoid any differences in the frequency of satellite association due to culture time,[11] all our cultures were harvested at 72 hours.

Results. Three groups of satellite association parameters were scored for each subject: A. The number of cells containing association per scored cells (N=25), the number of association per scored cells (N=25), the number of associated chromosomes per scored cells (N=25) (**Table 2**). B. The number of

Table 1 - Least significant differences for the parameters 1-3.

Parameter N Statistical Value	PI	P2	P3
O & C Difference*	12.72	17.48	35.75
LSD	1.69	2.56	5.20
A & C Difference	13.22	17.1	34.91
LSD	1.51	2.29	4.66
O & A Difference	0.5	0.38	0.84
LSD	1.89	0.90	5.84

N - number, LSD - (0.005) least significant differences, Difference* - the difference between 2 means, O - oligospermia, C - control group, A - azoospermia, P1 - the number of cells containing association, P2 - the number of associations for scored cells, P3 - the number of associated chromosomes for scored cells.

Table 2 - Least significant differences for parameters 4-5.

Parameter N Statistical Value	P4	P5
O&C Difference	16.43	0.7080
LSD	5.52	0.3527
A&C Difference	14.88	1.0909
LSD	2.26	0.3159
O&A Difference	1.55	0.3828
LSD	2.83	0.39

N - number, LSD - (0.005) least significant differences, O - oligospermia, C - control group, A - azoospermia, P4 - the number of small associations, P5- the number of large associations.

Table 3 - Least significant differences for parameters 6-8.

Parameter N Statistical Value	P6	P7	P8
O&C Difference	8.97	2.63	1.08
LSD	1.70	0.9793	0.2957
A&C Difference	9.99	2.78	0.48
LSD	1.52	0.8772	0.2649
O&A Difference	1.02	0.15	0.6
LSD	1.91	1.09	0.3316

N - number, LSD - (0.005) least significant differences, O - oligospermia, C - control group, A - azoospermia, P6- the number of cells with one association, P7 - the number of cells with 2 associations, P8 - the number of cells with more than 2 associations.

Table 4 - General f-test for the parameters 1-8.

Parameter N Statistical Value	P1	P2	P3	P4	P5	P6	P7	P8
F-cal.	238.92	184.577	185.934	156.26	29.855	128.230	32.111	33.418
F-crit	4.28	4.28	4.28	4.28	4.28	4.28	4.28	0.005
P*	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005

N - number, F-cal - F-calculated, F-crit - F-critical, P*- Probability, P1- the number of cells containing association, P2 - the number of associations for scored cells, P3 - the number of associated chromosomes for scored cells, P4 - the number of small associations, P5 - the number of large associations, P6 - the number of cells with one association, P7 - the number of cells with 2 associations, P8 - the number of cells with more than 2 associations, f-test - fisher test

small association (when 2 chromosomes are involved) and the number of large association (when more than 2 chromosomes involved) (**Table 3**). C. The number of cells with different number of association **(Figure 1)** (**Table 4**). In this instance, the cells were classified into 3 categories: 1. Cells with one association. 2. Cells with 2 associations. 3. Cells with more than 2 associations.

Considered as a whole, there was an astonishing increase in the number of satellite associations among the infertile patients in comparison to normal fertile control. By using the Fisher test (F-test) (**Table 1**), all the above mentioned parameters were highly significant ($p<0.005$) among the infertile patients in comparison to control group (**Table 1**). **Table 2** shows the over all differences of the first group (A) of satellite association parameters (as depicted before) between the infertile patients and the fertile control. It is clear that, in the first parameter (namely the number of cells containing association per scored cells, N=25), the infertile group had a significantly increased frequency of satellite association ($p<0.005$) compared to the control persons (**Table 2**). No significant differences were found among the infertile groups when compared statistically between them, although there was one exception. Again, parameter 2 (the number of association per scored cells, N=25) and parameter 3 (the number of associated chromosomes per scored cells, N=25) showed a significant increase in the infertile patients over those of normal control. **Table 3** displays the number of small association (parameter 4) and the number of large association (parameter 5). In both parameters the frequencies of satellite associations were much higher in the infertile men than normal fertile control. With regard to the cells with different number of associations namely (one, 2, and more than 2 associations). From **Table 4**, it could be noticed that there was a high increase in the number of cells with one, 2, and more than 2 associations in the form of infertile patients than those in the normal subjects. However, there was only one exception (**Table 4**) concerning parameter 8 (the number of cells with more than 2 associations), in which significant difference was noticed in oligospermia than those in azoospermia ($LSD_{0.005}$=0.33 16).

Discussion. Numerous studies concerning the association frequency of acrocentic chromosomes in somatic cells metaphases have been carried out.[16-18] Their aim was to specify the involvement of acrocentric associations in the occurrence of chromosomal non-disjunction and Robertsonian's translocation. A high frequency of specific acrocentic associations has been considered as a predisposing factor to meiotic and mitotic non-disjunction. Furthermore, the results of Mattei et al[19] also suggested that the unequal frequency observed in the distribution of Robertsonian's translocation constituted an argument supporting the view that the associations between acrocentric chromosomes do not occur at random.[20] Indeed, mitotic investigations were often inconsistent, some workers were reporting random association and some others the non-random involvement of acrocentric chromosomes.[21] It was

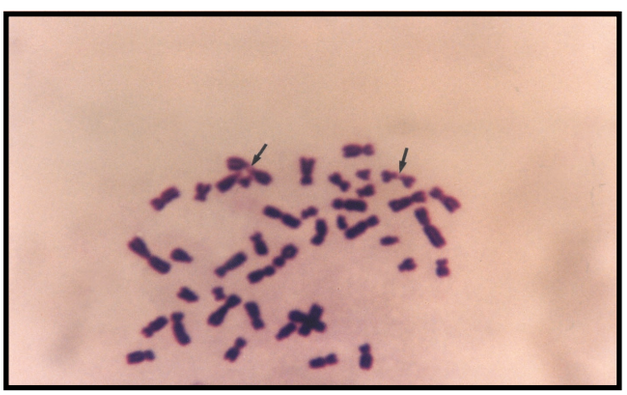

Figure 1 - This figure shows an acrocentric association between chromosomes 13,14,15 (D/D) and G/G chromosomes (21,22). The acrocentric lay within 1.2 μm of each other. The arrows indicate the associated chromosomes.

also suggested that the close proximity of short arms of specific chromosomes 13,14,15 (D-group) and chromosomes 21,22 (G-group) could explain the occurrence of exchange between them, leading to Robertsonian's translocation.[12] It appears that D/D translocation (13/14, 13/15 and 14/15) do not in any way need to be associated with phenotypic abnormality, except for a possible association with an increase in male sterility.[22] Accordingly, we have examined the frequency of satellite associations in mitotic preparations obtained from a group of the subfertile males. Our experience of associated behavior of acrocentric chromosomes in the infertile males is now related to 57 patients. Of those 57 patients, 24 patients were diagnozed with oligospermia and 33 patients diagnozed with azoospermia. The present study confirms the occurrence of a high fundamental increase in the 8 studied parameters (**Tables 2, 3, 4)** in infertile categories than those in controls. Indeed, those results may push us to suggest that the increasing frequency of satellite association in the infertile males may be considered as another indirect causal factor, which influence on spermatogenesis. On the other hand, the comparisons between the different groups of the infertile males (**Tables 2, 3, 4**), exhibit no significant differences, except parameter 8 which shows a significant difference between azoospermia and oligospermia (**Table 4**).

Two factors are known to play a causal role in the association of acrocentric chromosomes: 1. Nucleolar organizer regions (NOR) activity and 2. The presence of the satellite deoxyribonucleic acid (DNA) in the short arms of those chromosomes. It is well known that in the somatic metaphase there is a good correlation between the tendency for an acrocentic chromosome to associate and it's NOR activity measured by silver staining.[23] Moreover, a significant increase of silver-stained NOR in phytohemagglutinin (PHA)-stimulated lymphocytes has been investigated, there was the same strict correspondence between mitotic association of acrocentric chromosomes and silver staining technique (Ag-NOR staining). Other authors have suggested that the repetitive sequence plays a causal role in the heterochromatic attraction.[24] Indeed, quantitative evaluations of satellite DNA with different probes that had been carried out by Gosden et al[25] and Jeanpierre et al[26] supported this assumption. In fact, the tendency for specific acrocentric chromosomes to be in Robertsonian's translocation would result from a homology at a molecular level.[12] It has been demonstrated that breakpoints in Robertsonian's translocation are preferentially located within the satellite DNA, consisting of the short arms of acrocentric chromosomes.[27] Satellite DNA consists of various families repeated identical or close sequences. Each satellite DNA occurs one or more than one acrocentric chromosome.[28]

In conclusion, the model they proposed, based on homology at the molecular level, has the advantage of the reconciling the non-random distribution of Robertsonian's translocation. It is from the predilection here to emphasize the importance of mitotic analysis in the study of chromosomal behavior with regards to the phenomenon of acrocentric chromosome association. This study provides evidence indicating the involvement of factors predispose to Robertsonian's translocation. Furthermore, this study may, in conjunction with conventional cytogenetic and clinical investigations, shed some light on the basis of reduced reproductive fitness in the cases of infertile males.

References

1. Hansson A. Differences in the satellite association pattern in the human population. *Hereditas* 1970; 66: 21-30.
2. Hansson A. Satellite association in the human metaphase chromosomes. *Hereditas* 1971; 69: 269.
3. YingYip M, Fox DP. Relatives for acrocentric association frequency. *Hum Genet* 1981; 59: 245-249.
4. Rodman TC, Flehinger BJ, Squire RD. Patterns of association in the human metaphases complement: Ring analysis and estimation of associativity of specific chromosome regions. *Hum Genet* 1978; 41: 19-34.
5. Philips RB. Inheritance of acrocentric association patterns. *Humangenetik* 1975; 29: 309-318.
6. Nilsson C, Hansson A, Nilsson G. Influence of thyroid hormones on satellite association in man and the origin of chromosome abnormalities. *Hereditas* 1975; 80: 157-166.
7. Zang KD, Back E. Quantitative studies on the arrangement of human metaphase chromosomes. II. Influence of the preparation technique on the association pattern of the acrocentric chromosomes. *Cytogenetics* 1969; 8: 304-314.
8. Nankin HR. Satellite association in human lymphocytes and sodium citrate concentration of hypotonic (spreading) solution. *Can J Genet Cytol* 1971; 13: 115-118.
9. Nakagome Y. G-group chromosomes in satellite associations. *Cytogenet Cell Genet* 1973; 12: 336-341.
10. Curtis DJ. Acrocentric associations in mongol populations. *Humengenetik* 1974; 22: 17-22.
11. Mattevi MS, Salzano FM. Effect of sex, age and cultivation time on the number of satellites and acrocentric association in man. *Human Genetik* 1975; 29: 265-270.
12. Guichaoua MR, Devictor M, Harting M, Luciani JM, Stahl A. Random acrocentric bivalent associations in humanpachytene spermatocytes. Molecular implications in the occurrence of Robertsonian translocations. *Cytogenet Cell Genet* 1986; 42: 191-197.
13. Dumont MC, Bello MJ, Guichaoua MR, Luciani JM. Differential associative behavior of mitotic and meiotic acrocentric chromosomes. *Hum Genet* 1989; 82: 35-39.
14. Yasseen AA, McKenna PG. Cytogenetic evidence for hemizygisity at the thymidine kinase locus in P388 lymphocytes cells. *Experientia*1983; 39: 532-534.
15. McKenna PG, Yasseen AA. Hypersensivity of thymidine kinase deficient friend leukemia cells to the induction of cytogenetic aberration by mitomycin. *C Leukemia Researches* 1985; 9: 501-506.
16. Patil SR, Lubs HA. Non-random association of human acrocentric chromosomes. *Humangenetik* 1971; 13: 157-159.
17. Naylor AF, Warburton D, Warburton FE. Spatial relations of human chromosomes identified by quinacrine fluorescence at metaphase. II. Analysis of variance intercentromeric distances. *Humangenetik* 1973; 18: 307-313.

18. Galperin-Lemaitre H, Hens L, Kirsch-Volders M, Susanne C. Non random association of trypsin-banded human acrocentric chromosomes. *Hum Genet* 1977; 35: 261-268.
19. Ferguson-Smith MA. The sites of nucleolus formation in human pachytene chromosomes. *Cytogenetics* 1964; 3: 124-134.
20. Mattei JF, Ayme S, Mattei MG, Govvernet J, Giraud F. Quantitative and qualitative study of acrocentric association in (109) normal subjects. *Hum Genet* 1976; 34: 185-194.
21. Mattei MG, Soviah N, Aume S, Mattei JF, Giraud F. Variabilité interindividuelle des assoeration enter chromosome acrocentriques. *J Génét Hum* 1981; 29: 181-189.
22. Centerwall WR, Merrell PR. Familial D/D t (13q; 14q). *Clin Genet* 1975; 7: 91-97.
23. Miller DA, Tantravahi R, Dev VG, Miller OJ. Frequency of the satellite association of the human chromosomes is correlated with amount of the AG-staining of the nucleolus organizer region. *American Journal Genetics* 1977; 29: 490-502.
24. Schmid M, Vogel W, Krone W. Attraction between centrichetrochromatin of human chromosomes. *Cytogenet Cell Genet* 1975; 15: 66-80.
25. Gosden JR, Mitchell AR, Buckland RA, Clayton RP, Evans HJ. The location of four human satellite DNAs on human chromosomes. *Exp Cell Res* 1975; 92: 148-158.
26. Jeanpierre M, Weil D, Gallano P, Creav-Goldberg N, Junien C. The organization of two related subfamilies of the human tandemly repeated DNA is chromosome specific. *Hum Genet* 1985; 70: 302-310.
27. Gosden JR, Lawrie SS, Gosden CM. Satellite DNA sequences in human acrocentric chromosomes: information from translocations and hetromorphisms. *Am J Hum Genet* 1981; 33: 243-251.
28. Jones KW, Purdom IF, Prosser J, Corneo G. The chromosomallocalization of human satellite DNA I. *Chromosoma* 1974; 47: 161-171.