THE RELATION BETWEEN HUMAN Y CHROMOSOME MICRODELETIONS AND SPERM MORPHOLOGY

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ABSTRACT

Objective: Recent investigations have supported the importance of Y chromosome microdeletions in male infertility. Besides different factors, sperm morphology is also a very important component of the clinical evaluation of male fertility potential. The aim of our study was to understand the relation between Y chromosome microdeletions and sperm morphology in different risk groups.

Material and Methods: In this study, 34 infertile men were selected and examined in terms of sperm morphology and Y chromosome microdeletions. Semen analyses of the patients were performed by using Strict criteria and the samples were grouped according to the sperm abnormalities. Peripheral blood samples were examined for Y chromosome microdeletions by a multiplex polymerase chain reaction amplification of sequence-tagged sites (STS) of the Y chromosome.

Results: The incidence of deletions in the group was 14.7 % (5/34). The rates of Y chromosome microdeletion were 33.3% for megalohead, 33.3% for elongehead, 0% for roundhead, 100% for pinhead, 14.3% for severe teratozoospermia, 33.3% for severe neck abnormalities and 0% for tail-stump. Pregnancy could not be obtained from Y chromosome deleted cases.

Conclusion: It seems that especially patients with sperm head and neck abnormality have increased microdeletion risk. It is suggested that a detailed sperm morphology examination and classification is needed to clarify the Y chromosome deletion-sperm morphology relation.

Key Words: Y chromosome microdeletions, Sperm morphology, Male infertility

INTRODUCTION

Y chromosome is one of the sex chromosomes in males. It is known that this chromosome has an important role in spermatogenesis. A defect in any step of spermatogenesis can cause infertility (1-6).

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One of the important factors in spermatogenic failure is Y chromosome microdeletions (1,2,7-14). Although the relation between microdeletions and spermatogenic failure has been known for a long time, the defective regions were identified at the molecular level only in recent years. There are a total of four AZoospermic Factor (AZF) regions on the Y chromosome AZFa, AZFb, AZFc and AZFd (1-12). These deletions in the Yq11 region are mostly undetectable by cytogenetic analysis. Molecular analysis can detect the presence/absence of these deletions by polymerase chain reaction (PCR). In spite of this, there is no agreement about which factors play a role in male infertility due to unknown causes. Different studies on male infertility cases showed a wide range (1%-55%) of Y chromosome microdeletion rates (3,15-32). It is suggested that different factors related to the usage of different techniques can affect these rates.

Since the deleted regions involve the genes which are responsible for sperm production, sperm number, structure and function can be affected (17-22,33,34). The association between the phenotypic characteristics of sperm and the genetic structure of Y chromosome is being examined by ongoing studies. Especially, after understanding that Y chromosome microdeletions can be a factor in infertile men and they can be transmitted by ICSI from father to son, more interest has been focused on deletion and sperm parameters including morphology, number and function (35-39). However, the possible relation between Y chromosome microdeletions and sperm morphology is not yet clear.

The goal of the study was to determine the sperm morphology and Y chromosome microdeletion correlation in order to understand its influence on male infertility.

MATERIALS AND METHODS

Thirty-four infertile men with sperm morphology defects who referred to Istanbul Memorial Hospital IVF Center were selected for this study. Semen analysis was done by using WHO and Kruger Strict Morphology Criteria and samples were grouped according to sperm abnormalities.

Semen Analysis

Sperm concentration and movement characteristics were detected by Makler counting chamber. The evaluation of motility and progression was performed by counting at least 100 sperm under the light microscope. Kruger Strict Morphology Criteria were used to evaluate sperm morphology by light microscopy.

DNA Isolation and Polymerase Chain Reaction

After the isolation of DNA from peripheral blood by using Promega Wizard Genomic DNA Purification Kit, Promega Deletion Detection Kit Version 1.1 was used to detect 18 loci on Y chromosome by multiplex polymerase chain reaction.

Analyses of PCR products were performed by agarose gel electrophoresis.

RESULTS

The results of the sperm analysis of 34 patients are shown in Table I. As can be seen from the table, apart from two cases, all the cases were found to have low sperm number. In addition, it was found that the sperm volume and the sperm numbers did not correlate. Although all samples had different structural sperm defects, Y chromosome microdeletion was found in only 5 patients. Twelve patients gave healthy birth by the help of assisted reproductive techniques.

Y chromosome microdeletion rates in pinhead, (megalohead-elonge head and severe neck abnormality) and severe teratozoospermia were 100%, 33% and 14% respectively (Table II). No microdeletion was detected in tail-stump and round head abnormalities.

The products of multiplex PCR were evaluated by using positive and negative controls on agarose gel electrophoresis (Fig 1).

DISCUSSION

Recent studies have confirmed the importance of Y chromosome microdeletions in male infertility (1,2,7-14,31,40). However, genotype-fenotype correlation was not clearly explained in these

Table I: The clinical and molecular findings of 34 infertile men

SAMPLE No	AGE	SEMEN VOLUME (ml)	SPERM NUMBER (/ml)	MOTILITY (%)	SPERM MORPHOLOGY	Y CHR. MICDEL	NORMAL BIRTH
1	34	6	6 million (Oligozoospermic)	50	Roundhead, Severe neck abnormality	-	-
2	36	2,5	22 million (Normozoospermic)	10	Round head	-	-
3	28	4,5	30.000 (Oligozoospermic)	20	Severe teratozoospermia	-	+
4	35	3	8 million (Oligozoospermic)	52	Round head	-	+
5	39	2	26.000 (Oligozoospermic)	12	Severe teratozoospermia	-	+
6	43	3	9 million (Oligozoospermic)	13	Round head	-	+
7	37	3,5	49 million (Normozoospermic)	34	Elongehead, Severe neck abnormality	-	+
8	21	1,5	200.000 (Oligozoospermic)	32	Megalohead, Pinhead	+	-
9	30	1	3000 (Oligozoospermic)	11	Severe teratozoospermia	-	-
10	36	3	17 million (Oligozoospermic)	11	Megalohead, Elongehead	-	+
11	31	1	1000 (Oligozoospermic)	0	Megalohead, Elongehead, Pinhead	+	-
12	34	1,5	700.000 (Oligozoospermic)	42	Megalohead, Elongehead	-	-
13	37	3	30.000 (Oligozoospermic)	63	Megalohead, Roundhead	-	+
14	23	2	10.000 (Oligozoospermic)	30	Megalohead	-	-
15	28	1	Azoospermic	-	Severe teratozoospermia	-	+
16	35	3	6 million (Oligozoospermic)	30	Round head	-	+
17	53	3	4 million (Oligozoospermic)	31	Severe teratozoospermia	-	-
18	45	3	3 million (Oligozoospermic)	13	Severe teratozoospermia	+	-
19	29	1,5	700.000 (Oligozoospermic)	<1	Severe teratozoospermia	-	-
20	38	4	7 million (Oligozoospermic)	14	Severe teratozoospermia	-	+
21	31	3	400.000 (Oligozoospermic)	33	Severe teratozoospermia	-	-
22	39	2,5	700.000 (Oligozoospermic)	14	Severe teratozoospermia	-	-
23	39	2	2 million (Oligozoospermic)	10	Severe teratozoospermia	-	-
24	29	3	2 million (Oligozoospermic)	20	Severe teratozoospermia	-	-
25	32	2	300.000 (Oligozoospermic)	5	Severe teratozoospermia	-	+
26	25	5	500.000 (Oligozoospermic)	2	Round head	-	+
27	31	3	200.000 (Oligozoospermic)	40	Round head	-	-
28	25	3	600.000 (Oligozoospermic)	5	Round head	-	-
29	25	3	156.000 (Oligozoospermic)	23	Elongehead	-	+
30	34	2,5	600.000 (Oligozoospermic)	16	Severe neck abnormality, Elongehead	+	-
31	35	1	2000 (Oligozoospermic)	0	Tail-stump	-	-
32	32	3	5.5 milyon (Oligozoospermic)	1	Tail-stump	-	-
33	32	4	Azoospermic	0	Tail-stump	-	-
34	38	3	3000 (Oligozoospermic)	3	Severe teratozoospermia	+	-

Table II: The abnormalities of sperm mophology and Y chromosome microdeletions.

	Megalohead	Elongehead	Roundhead	Pinhead	Severe teratozoospermia	Severe Neck abnormalities	Tail-stump
Total Number of Samples	6	6	9	2	14	3	3
Y Chromosome Microdeletion Positive Samples	2	2	0	2	2	1	0
Y Chromosome Microdeletion Rate (%)	33,3	33,3	0	100	14,3	33,3	0

studies. Only the relation between certain microdeletions and numerical sperm abnormalities such as azoospermia and oligozoospermia was tried to be examined. In this study we found Y chromosome microdeletions in five oligozoospermic patients (15%). In accordance with previous studies (20,23,37,41-43), one patient out of five exhibited total deletion of AZF region, while the remaining four patients showed AZFc deletions.

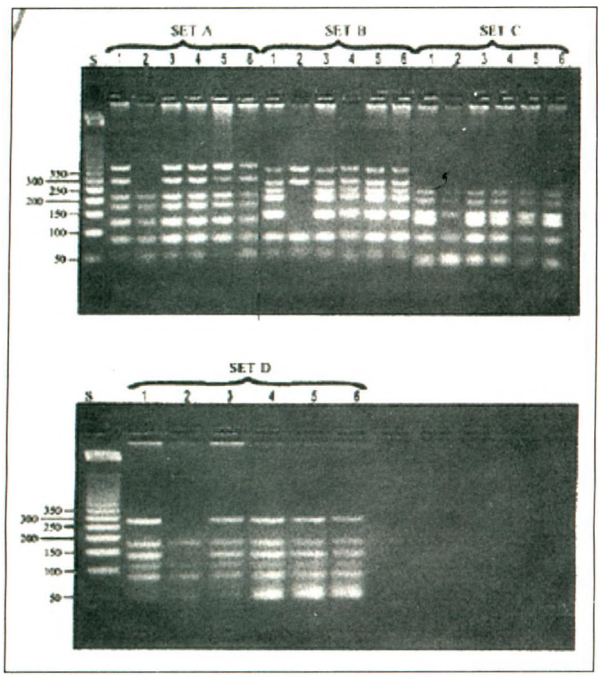

Fig. 1: Agarose gel electrophoresis of multiplex PCR (in four sets) products correspond to oligozoospermic or azoospermic patients (lanes: 2,3,4.5 and 6) and normal control (lane 1). S= size marker. Deleted regions can be seen easily in patient no:2.

In assisted reproduction units, Y chromosome microdeletion detection is commonly applied for azoospermia and oligozoospermia in infertility cases. The other parameters of sperm analysis such as functional and structural defects are not generally considered as test criteria. Shortly, structural sperm defects which are accepted as a test criteria in this study are not used for routine deletion analysis.

Because of keeping the numerical defects of the sperm in the foreground during examination and due to insufficient structural evaluation, the reliability of the spermiogram results can be affected. Morphology can be accepted as a test criteria for deletion detection whenever the relation between sperm structure and deletion becomes clear.

In our study group with structural sperm abnormality, the microdeletion rate is 15% supporting the genotype-phenotype correlation. This result is in the range of the previous studies (1% -55%) which were designed generally according to the numerical criteria in infertile men (3,15-32).

The relation is not clear when we take three parameters (Y chromosome microdeletions, numerical and structural abnormalities) into account but it is found that in this preliminary group Y chromosome microdeletion rates are higher in sperm head and neck abnormalities. Especially when we take the pinhead group into account, both samples are Y chromosome deleted and this data seems to give an idea for future studies as pinhead is not so frequent as the other head abnormalities. Although these preliminary findings suggest that there can be an association especially between these two morphological abnormalities and Y chromosome microdeletions, a detailed structural sperm examination and more samples are needed to obtain a significant conclusion.

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