

The significance of Y chromosome microdeletion analysis in subfertile men with clinical varicocele

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Abstract

Introduction: The aim of study is determining the cost-effectiveness of detection analysis in the presence of exceptional patients who have mild semen disorders, and beware of unnecessary varicocele repairs; and to ascertain whether patients with clinical varicocele should undergo Y chromosome (Yq) microdeletion analysis as a routine procedure.

Material and methods: Varicocele with reflux was diagnosed in 51 male patients with subfertility symptoms upon physical examination (PE), confirmed by scrotal colour-Doppler ultrasound (CDU). After cytogenetic examination, Yq microdeletion analysis was performed on the peripheral blood samples using Promega Y Chromosome Deletion Detection System Version 2. Varicocele repair was performed under general anaesthesia with optical magnification (3-fold) through a subinguinal approach.

Results: The mean age of the patients was 27.9. Values of semen concentration ranged from 0 to 72 million/ml, motility from 0 to 65% (A + B) and Kruger from 0% to 18%. The PE revealed normal size and consistency in the bilateral testicles. All patients were cytogenetically normal. However, Yq microdeletion was detected in 2 patients, 1 with mild oligoteratozoospermia and partial AZFb deletion (sY121) and the second patient with severe oligozoospermia and partial AZFc deletion (sY254 and sY255), and they were not subjected to varicocelectomy.

Conclusions: The routine performance of pre-operative Yq microdeletion analysis in patients with clinical varicocele does not seem to be cost-effective but the omission of patients with mild oligozoospermia would have subjected them to an unnecessary varicocelectomy and/or further ICSI applications and also would have caused the failure of referral for genetic counselling.

Key words: Y chromosome microdeletion, subfertility, varicocele.

Introduction

Subfertility is defined by the World Health Organization (WHO) as failure to conceive over 12 months of unprotected frequent intercourse and affects approximately 15% of couples; among these half are male-related [1]. Varicocele is a physical abnormality present in 11% of adult males [2] and in 25% of those with abnormal semen analysis [3]. Varicocelectomy, in general, brings an improvement in semen parameters in 50-80% of patients and pregnancy rates vary from 20% to 69% [4].

One of the most significant pathogenetic defects associated with male infertility is microdeletions of the long arm of Yq. 13% of azoospermic men, 1-7% of severely oligozoospermic men, and 5% of men with severe primary testicular failure and with a sperm density of less than 5 million/ml showed Yq microdeletion [5]. On the basis of selection criteria of the patients, the rate in the infertile population is in the range 1-55% [6, 7]. The difference in Yq microdeletion type and their frequency in different reports may reflect variations in the sample group and selection of specific sequence tagged site (STS) markers [8]. Ordinary clinical parameters, such as hormone level, testicular volume, cryptorchidism and infections do not have an estimated value [9, 10].

Men who have varicocele and a sperm count less than 5 million/ml must be evaluated for presence of genetic abnormalities because they appear to have a poorer response to varicocele repair than men without coexisting genetic lesions [4].

In this study we aimed to: determine the Yq microdeletion prevalence among men with varicocele-related subfertility; compare the cost-effectiveness of detection analysis and varicocele repair in the presence of exceptional patients who have mild semen disorders, and beware of unnecessary varicocelectomies and consequently to avoid prolonged treatment of subfertility; ascertain whether patients with clinical varicocele should undergo Yq microdeletion analysis as a routine procedure.

Material and methods

The medical records of 51 consecutive men with subfertility and clinical palpable varicocele who had been admitted to our clinic between September 2006 and October 2008 were reviewed. All patients were primary subfertile and had at least a 1-year history with the current partner. Males with normozoospermia, subclinical varicocele, secondary infertility and having additional female factors in the aetiologies were excluded from the study.

These men were subjected to comprehensive questionnaires related to their medical, surgical, sexual and family histories, and lifestyle habits. Furthermore, a comprehensive systemic urogenital examination was performed. Varicocele examination was performed on the patient in a seated position or standing erect prior to and after the Valsalva manoeuvre and was categorized as grade 1 (palpable only during the manoeuvre), grade 2 (palpable without the Valsalva manoeuvre) or grade 3 (dilated veins visible) [11]. Bilateral varicocele was observed in 3 of 51 patients. Preoperative varicocele grades were 1 in 16 patients, 2 in 15 patients and 3 in 17 patients with left unilateral disease. In the patients with bilateral varicocele, grades were 1 in 2 patients and

3 in 1 patient on the left side and 1 in 2 patients and 2 in 1 patient on the right side. Colour-Doppler ultrasound (CDU) was performed to detect refluxes and to confirm varicocele diagnosis. To define a varicocele detected ultrasonographically, we used a 2 mm cut-off for vein diameter and prolonged reflux longer than 1 s [12]. At least 3 semen samples were obtained from each patient at least 2 weeks apart and following sexual abstinence for 2-5 days. Sperm concentration and motility were assessed according to the WHO criteria [13] and sperm morphology was assessed according to Kruger's strict criteria [14] in the same andrology laboratory in a blind fashion.

After cytogenetic examination, Yq microdeletion analysis was performed on the peripheral blood samples collected from 51 patients, using Promega Y-Chromosome Deletion Detection System Version 2.0 (Promega Corporation—2800 Woods Hollow Road—Madison, WI 53711 USA). This system provides a rapid method for detecting deletions that occur in YqAZF, consisting of 20 primer pairs that are homologous to previously identified and mapped STS [8]. These primers amplify non-polymorphic short DNA segments from the Yq when used in polymerase chain reactions (PCR). This makes it possible to determine the presence or absence of all STS by performing 5 parallel PCR amplifications. Yq deletions in the regions that are amplified by these primer sets have been associated with male infertility [15, 16].

Four of the Multiplex Master Mix sets (A-D) contain a control primer pair that amplifies a fragment of the X-linked SMCX *locus*. The fifth Multiplex Master Mix set (E) contains a primer pair that amplifies a unique region in both male and female DNA (ZFX/ZFY). These control primer pairs are internal controls for the amplification reaction and the integrity of the genomic DNA sample. Finally, Multiplex E includes a primer pair that amplifies a region of the SRY gene. This is a control for the testis determining factor on the short arm of the Yq and allows XX males arising from Y to X translocations to be detected.

Varicocele repair was performed under general anaesthesia with optical magnification (3-fold) through a subinguinal approach preserving the arteries and lymphatics.

Informed written consent was obtained from each subject. After a thorough clinical evaluation, a respective diagnosis of subfertility was given to each individual accordingly and genetic counselling has been provided to the patients. This research was conducted in accordance with the Helsinki Declaration principles.

Results

The mean age of the patients was 27.9 years (18-38 years). The sperm concentration ranged from

Table I. Preliminary clinical data of semen parameters in accordance with WHO criteria and Kruger's strict criteria

Patient classification	n	%
Azoospermia	4	7.8
Oligoasthenoteratozoospermia (OAT)	15	29.4
Oligoteratozoospermia	5	9.8
Oligoasthenozoospermia	2	3.9
Asthenoteratozoospermia	11	21.6
Asthenozoospermia	1	2.0
Teratozoospermia	13	25.5
Total	51	100

0 to 72 million/ml, the motility (A + B) from 0 to 65% and Kruger from 0% to 18%. In physical examination, bilateral testes were of normal size and consistency and after the Valsalva manoeuvre the mean vein diameter changed from 2.37 ±0.54 to 2.79 ±0.58 according to the rest position in CDU. The category and rate of patients according to seminal analysis are summarized in Table I. All patients were cytogenetically normal. In our patient cohort of

51 individuals, Yq microdeletion was detected in 2 patients (3.92%). The first patient was 24 years old and had grade 1 varicocele with reflux on the left side. Values of semen volume (ml), sperm concentration (million/ml), motility (A + B) and Kruger were 3.5, 15, 25 + 19 and 2, respectively. There were no pathologies in cytogenetic examination (Figure 1A). However, microdeletion was detected in sY121 of the AZFb region of the Y chromosome (Figure 1B). The second patient was 23 years old and had grade 1 varicocele on the left side. The semen volume was 2 ml and there was only one sperm in each microscope area. No pathologies were detected in cytogenetic examination (Figure 2A). Microdeletions were present in sY254 and sY255 in the AZFc region of the Y chromosome (Figure 2B).

Varicocelectomy was applied to all patients except for those two 2 patients who had partial AZF deletions.

Discussion

The first cases of Yq microdeletions and male infertility were reported in 1992 [17] and many case series have subsequently been published. Yq

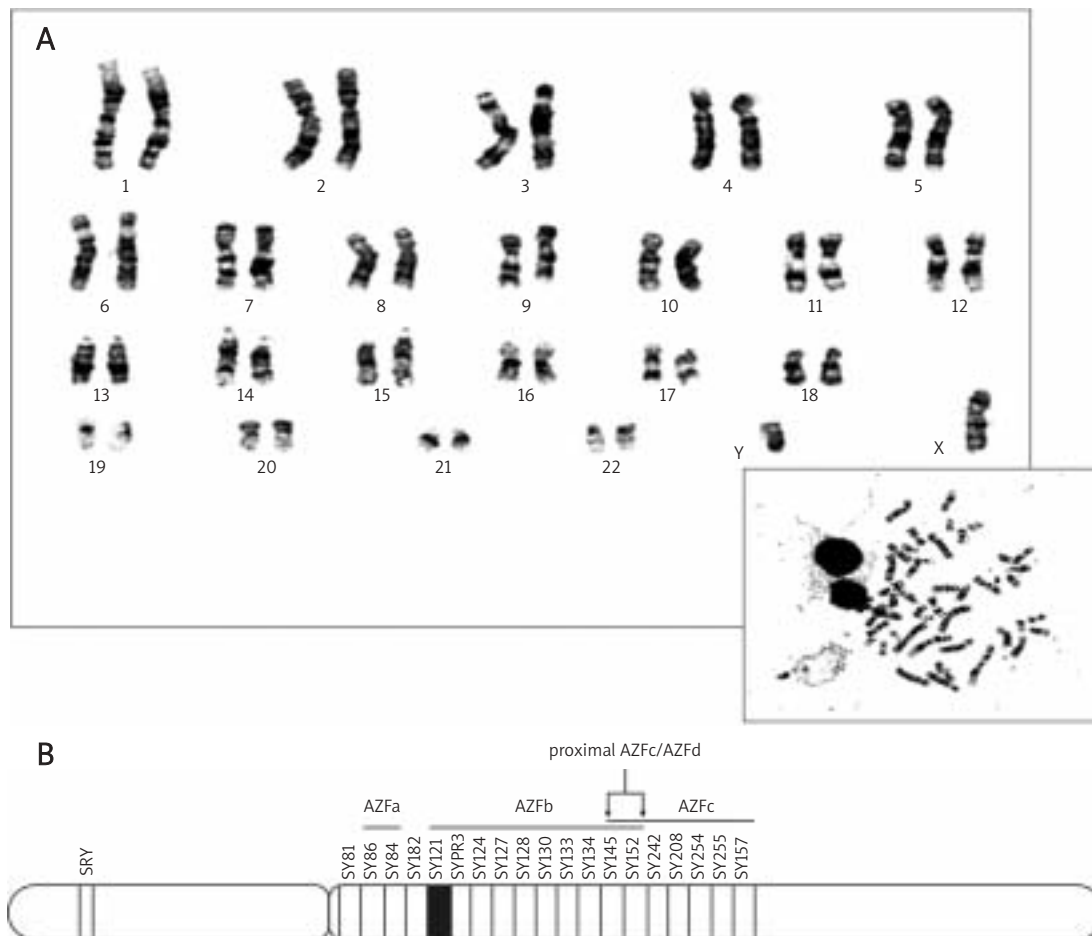


Figure 1. The patient who had a deletion on the AZFb region, sY121 locus: **A** – cytogenetic formation, **B** – Yq map

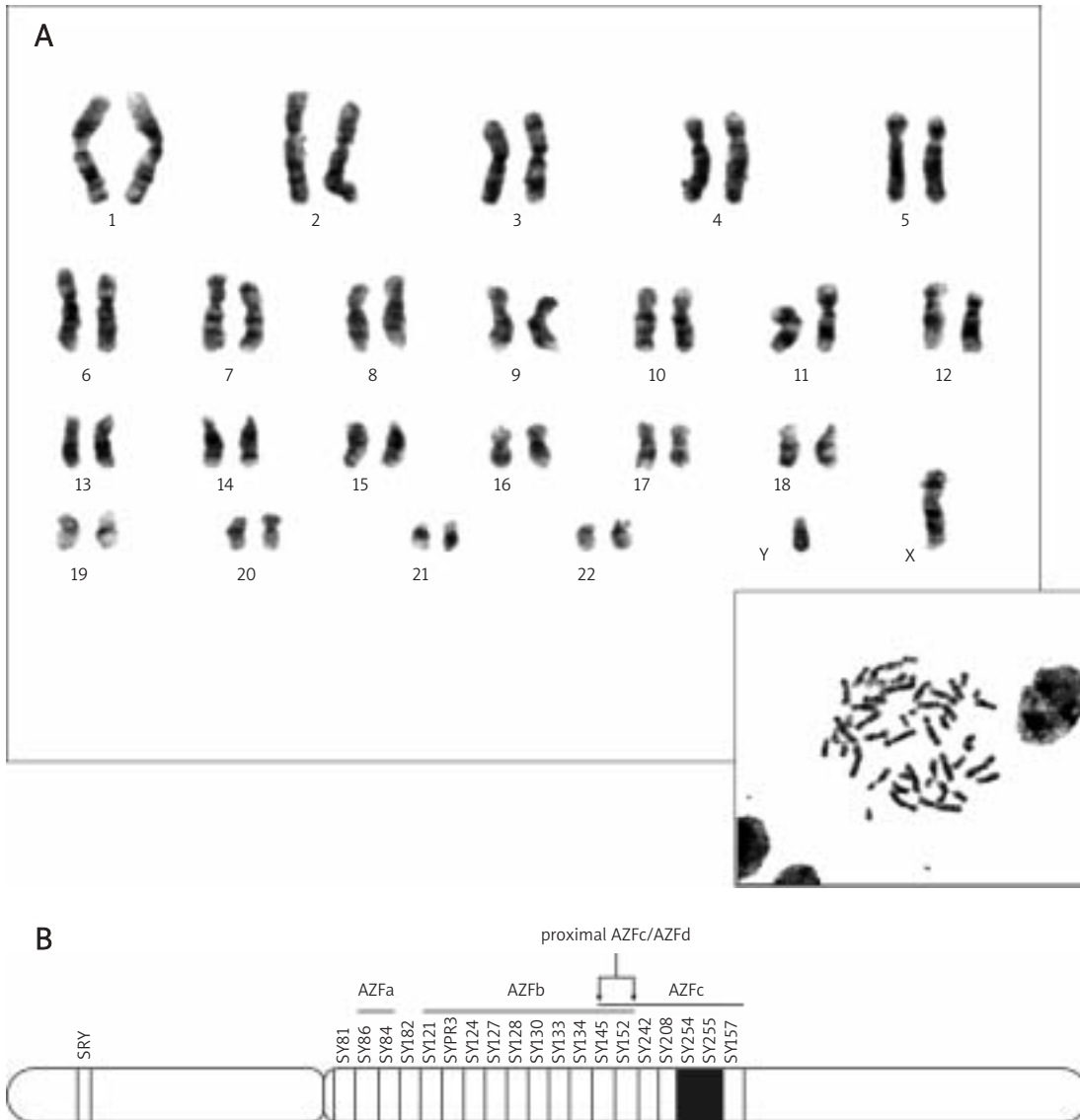


Figure 2. The patient who had deletions on the AZFc region, sY254 and sY255 locus: **A** – cytogenetic formation, **B** – Yq map

microdeletions play a role in 0.3-7% of infertile cases in connection with the male factor and this rate is higher in a selected population with severe seminal parameter disorder [6, 7, 16]. In Yq microdeletion cases, AZFc region deletion is the highest group with 60%, followed by AZFb, AZFbc, AZFa and AZFa + b + c at 16%, 8%, 5% and 4%, respectively.

The deletion of the AZFa region in clinical cases causes mostly azoospermia, whereas the deletions of AZFb and AZFc regions mostly cause oligozoospermia or teratozoospermia [16, 18, 19]. In non-obstructive azoospermia cases with deletions in the AZFa region, until today, no sperm could be found even with testicular sperm extraction (TESE [20, 21]). If the deletion in the AZFb region is sufficiently large, this may cause azoospermia and again the chance of finding sperm even with TESE is very slim. While there is a chance

to provide sperm with TESE in partial AZFb deletions, in almost half of the cases, the chance of presence of mature spermatozoa is almost non-existent in cases with complete AZFb deletion [22]. In azoospermic males with AZFa and AZFb deletion, although successful results during sperm collection with TESE have been obtained, in 38% of infertile males with deletion in the AZFc region, sperm has been detected with sperm analysis [20].

Varicocele has been detected in 25% of those males who have anomalous seminal parameters [23]. There is a high incidence of chromosomal defects in severely oligozoospermic men compared with mildly oligozoospermic men among men with varicocele-related infertility, idiopathic infertility and overall male infertility, which is not the same in the case of Yq microdeletions. There was no significant difference in the cumulative frequencies of Yq

microdeletions in individuals with varicocele and idiopathic infertility. No correlation was seen between the sperm concentration and the size or the extent of the microdeletions [24].

In very few of the many studies dealing with Yq deletion have patients with varicocele been included or, in general, have patient populations with azoospermia and severe oligozoospermia been evaluated [25, 26]. Moro *et al.* did not detect any deletions in a patient group of 80 individuals with varicocele and mild oligozoospermia, but they reported 17.5% Yq microdeletion in the second patient group consisting of 40 individuals with severe oligozoospermia and idiopathic infertility [27]. Rao *et al.* found Yq microdeletion in 3 of 57 patients (5.26%) with non-idiopathic infertility. Of these patients 1 has been diagnosed with azoospermia, another 1 with mild oligozoospermia, and the last 1 with severe oligozoospermia [24]. Dada *et al.* observed microdeletion in 7 of 72 infertile patients (9.7%) with varicocele; 4 of these patients were diagnosed with azoospermia and 3 with severe oligozoospermia [28].

Numerous studies have emphasized that the performance of Yq microdeletion analysis for varicocele patients, diagnosed with azoospermia and severe oligozoospermia (having sperm concentrations lower than 5 million/ml), was important as a diagnostic and prognostic factor [10, 25-28]. In this study, we included patients who had applied to our clinic with the complaint of subfertility and who had been diagnosed with clinical varicocele. We did not eliminate mild oligozoospermic patients (sperm count 5-20 million/ml). In our patient cohort of 51 individuals, Yq microdeletion was detected in only 2 patients. In our first patient with mild oligoteratozoospermia, partial AZFb deletion (sY121) and in our second patient with severe oligozoospermia, partial AZFc deletion (sY254 and sY255) were detected and according to best practice guidelines [29] for Yq microdeletion studies when both markers of sY254 and sY255 were deleted, a diagnosis of complete deletion of the AZFc region could be made. Moreover, the literature suggests that patients with varicocele and genetic infertility do not benefit from varicolectomy [4, 25]. These 2 patients were consequently not subjected to varicolectomy.

In most andrology and infertility centres in Turkey and abroad, the Yq microdeletion assay is performed for infertile males whose sperm density is below 5 million/ml. This is an essential prerequisite for infertile men undergoing intracytoplasmic sperm injection (ICSI) to rule out the possibility of transmission of the same deletions to their male offspring, who could also experience infertility. This fact is also significant because these men are at an increased risk for having recurrent

miscarriages with their partner, and children with birth defects and learning disabilities.

To enable decision-making, patients need to be informed about the tests. If testing is performed and an abnormality is identified, professional genetic counselling should be offered, where patients are provided with an explanation of the cause of the genetic defect they have been identified with by a geneticist. More importantly, the consequences for the person tested, his future children and his family members are discussed and, if necessary, further counselling and testing for other family members is organised. Following the explanations made to the families, approximately 21% of couples with microdeletions have given up ICSI [30].

The package cost of Yq microdeletion analysis in our country is approximately \$470. The cost of single-sided varicolectomy with optical magnification to the Social Security Institution is approximately \$500. According to our data, since the rate of Yq microdeletion, in our cohort, consisting of patients with subfertility and clinical varicocele, is low (3.92%), routine performance of this analysis in such a patient population did not appear to be cost-effective. However, in our patient cohort, since Yq microdeletion was determined in 1 patient with mild oligozoospermia, as reported in the study of Rao *et al.* [24], but different from many studies [25-28], the failure to perform this analysis will cause the patient to fail to obtain genetic counselling and subject them to an unnecessary surgical procedure. However, considering our cohort of 51 patients and the other studies mentioned above, cohorts of sufficient size to recommend this analysis as a routine procedure prior to varicolectomy do not exist.

In conclusion, Yq microdeletions contribute only marginally to the totality of human male infertility, but when present, the introduction of ICSI as an artificial reproductive technique (ART) may allow for the transmission of such mutations to the next generation [31]. Our data suggest that the routine performance of Yq microdeletion analysis in patients with clinical varicocele does not appear to be cost-effective, but there are several considerations that support a routine assessment of Yq deletions. Firstly, a positive test would provide a firm diagnosis of the man's problem, which, for some couples with longstanding infertility, can help resolve stress, blame or feelings of guilt. Secondly, knowledge of the type of Yq deletion may assist the clinician in determining the best type of ART treatment. Thirdly, couples should be offered this information, as they must understand that their male offspring will almost certainly be subfertile and require reproductive monitoring from the time of sexual maturation. However, as stated in the

literature, most genetic defects have been found in patients with azoospermia or sperm concentrations of 5 million/ml or lower and the assay was suggested for application only to this group of patients, but our findings support that the omission of patients with mild oligozoospermia would have subjected them to an unnecessary varicocelectomy and/or further ICSI applications and also would have caused the failure of referral for genetic counselling. Within this framework, large-scale studies are needed.

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