Cytogenetic and Y Chromosome Microdeletions Analyses for a Cohort of Palestinian Oligozoospermic and Azoospermic Infertile Men

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Abstract

Genetic causes account for roughly 15% of male infertility in most Western countries. Klinefelter Syndrome and submicroscopic deletions on the Y chromosome involving AZF region are the most common genetic causes of male infertility associated with azoospermia and oligozoospermia. The aim of this study was to assess the frequency of chromosomal aberrations and Y chromosome microdeletions in a group of azoospermic and oligospermic infertile men in the southern part of the West Bank (Occupied Palestinian Territories). Twenty-seven samples from Hebron and Bethlehem were analyzed by standard G-banding techniques for numerical and structural chromosomal rearrangements. Twenty-six samples were analyzed for Y chromosome microdeletions by multiplex PCR using primers for AZFa, AZFb and AZFc regions. The results showed chromosomal abnormalities only in azoospermic males with 14 patients having abnormal karyotype (25% of all patients 37.8% of azoospermic patients); 11 had non-mosaic 47,XXY, one mosaic 47,XXY/46,XY, one mosaic 45,X/46,XY, and one 48,XXXY. The present study revealed a higher incidence of chromosomal aberrations than reported for Western populations. None of the subjects was positive for deletions of the AZF regions tested. Another study from Gaza also showed absence of Y chromosome microdeletions in Palestinians. The combined data validate the need for cytogenetic analyses for diagnosis and genetic counseling for infertile Palestinian azoospermic men but that classic microdeletion analysis can be of limited use in this population.

Keywords: Male Infertility, Occupied Palestinian Territories, Klinefelter Syndrome, Y-Chromosome Microdeletions.

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Introduction

Infertility is a serious problem around the world encountered in about 15% of married couples[1]. Male factor infertility is a significant contributing factor and is caused by genetic factors in up to 15% of cases [2, 3]. The most common genetic factors causing male infertility are Klinefelter syndrome and, secondarily, Y chromosome microdeletions [3]. Klinefelter syndrome prevalence among oligospermic men was reported at 1.5% and in azoospermic men at 13% [4, 5]. In azoospermic Klinefelter patients, the possibility of finding sperms by testicular biopsy is around 50%, and can reach up to 70% by microdissection TESE (testicular sperm extraction) which opened possibilities for infertility treatments via ICSI[6, 7].

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The incidence of Y chromosome microdeletions was reported at 10% of American azoospermic and oligospermic infertile men [8]. The critical region responsible for spermatogenesis was named AZF coding for three critical azoospermia factors: AZFa, AZFb and AZFc [9, 10]. A complete deletion of AZFa is characterized by a testes histology of Sertoli cell only (SCO) and azoospermia, and implies the impossibility of finding sperms in testes [11]. Complete deletion of AZFa or AZFb is characterized by a histological picture of SCO or spermatogenic arrest, and also implies the virtual impossibility of finding sperms in testes [12]. Partial deletions of AZFb and AZFc are associated with sperm retrieval of 50% of cases[13]. Complete deletion of AZFc is the most common deletion, and can cause different degrees of spermatogenic failure, which range from absence of germ cells in testes to presence of sperms in ejaculate (oligozoospermia). Partial deletions within AZFc can occur, and different partial deletions were reported with variable phenotypes, ranging from moderate oligozoospermia to azoospermia, and there is a major controversy on their real impact on male fertility[14, 15]. In azoospermic AZFc-deleted men, the possibility to find sperms in testes is about 67%[16] so they can undergo ICSI and have a biological offspring[17, 18].

Taken together, these studies show the importance of genetic analysis of infertile men using chromosome studies and for microdeletions of Y chromosome. However, most studies are done in European and other Caucasian populations with few studies done in developing countries. In Palestine, there was one study in the Gaza Strip on Y chromosome microdeletion analysis which produced surprisingly negative findings[19]. We performed cytogenetic analysis and Y chromosome microdeletions in a cohort of infertile men from the West Bank (Palestinian areas) in order to add to the literature on causes of infertility in developing countries and specifically in Palestine.

Material and Methods

Study population: A total of 56 Palestinian infertile patients (37 azoospermic and 19 oligozoospermic) were recruited from assisted reproductive centers and private infertility clinics from June 2010 to June 2013 and evaluated for chromosomal abnormalities and Y chromosome microdeletions. Institutional approval and informed patient consent were received in compliance with ethics guidelines. Patients came from Bethlehem and Hebron governorates. Patients and doctors supplied infertility history and semen analysis tests. Oligozoospermic patients had less than 5 million sperm/ml in semen analysis. A urologist examined patients to ensure absence of physical causes or other non-genetic causes of infertility.

Cytogenetic Analysis: The blood samples were drawn into 10 ml sodium heparin tubes. 0.5 ml of blood was cultured in media with phytahaemagglutinin for three days (traditional cytogenetic culture medium Sigma Aldrich). Harvest and GTG-banding was done by classical cytogenetic methods. A minimum of 20 metaphases were examined and when these are normal, thirty more cells were examined for sex-chromosome abnormalities to rule out mosaicism. From each case, documentary photographs were taken on an Olympus microscope BX41 (Olympus Company) and karyotyped via Cytovision software (Cytovision, Applied Imaging Inc.).

Molecular Analysis: DNA was extracted by salt based method. Two multiplex PCR was run for each patient two times, each multiplex contained 3 primers for the three AZF regions (AZFa, AZFb, and AZFc) and 2 internal controls (ZFY and SRY). The STS (sequence-tagged sites) primers for AZF regions are sY84, sY86 for AZFa, sY127, sY134 for AZFb and sY254, sY255 for AZFc. These

protocols follow existing published methods and comply with established best practices guidelines[20].

Follow-up Counseling and Additional Data Collection: Patients were counseled appropriately concerning their results following standard protocols approved by the European and American boards of medical genetics and relevant literature to their abnormalities (for example straight 47, XXY Klinefelter, variants, and mosaics). In some cases we sought additional medical information for unusual cases (see discussion).

Results

We examined 37 azoospermic and 19 oligozoospermic infertile men (total 56) for chromosomal abnormalities. Chromosome abnormalities were found only in azoospermic men. Fourteen (25% of all patients or 37.8% of azoospermic patients) had chromosome abnormalities: 11 men had 47,XXY, 1 had 48,XXXY, one was mosaic 46,XY/47,XXY, and one mosaic 45,X/46,XY (Table 1). No patients had other structural or numerical abnormalities by G-banding at 450-600 band resolution.

Table 1. Results of Cytogenetic Analysis

	Azoospermia	Oligospermia
Patients	37	19
46,XY	23	19
47,XXY	11	0
48,XXXY	1	0
47,XXY/46,XY	1	0
45,X/46,XY	1	0

Twenty azoospermic and nine oligospermic infertile men (total 29) were analyzed for Y chromosomal microdeletions using 8 STSs approved by EAA/EMQN best practice guidelines[20]. No subject had deletion for any of these STSs (sY14 (SRY), ZFX/ZFY, sY84, sY86, sY127, sY134, sY254, sY255).

Discussion

The prevalence of chromosomal aberrations ranges from 4.3% to 17.5% in azoospermic and oligozoospermic infertile men attending reproductive centers[2, 22-26]. In our case it was even at a higher level (25% of all cases or 37.8% of azoospermic cases). Assuming average of 10% of patients studied in Europe and North America with oligo or azoospermia to have Klinefelter or variants of Klinefelter, and a binomial distribution, the test statistic would be 1.99, with associated P value of 0.024 and we reject the null hypothesis that our proportion of cytogenetic abnormalities is not different from the reported mean of 10%. The high frequencies of chromosomal aberrations may reflect geographic variations or different patients' selection criteria. Chromosomal abnormalities are more prevalent in azoospermic than oligozoospermic infertile men in other studies but in our case not one of the 19 oligozoospermic men showed a cytogenetic abnormality. This could be sample-size related issue and we would need to examine a larger series of oligozoospermic Palestinian males.

We found one mosaic Klinefelter patient (47,XXY/46,XY) with azoospermia. Another mosaic patient was mosaic for 45,X/46,XY. Such mosaicism in males is rare and they show short stature and occasional azoospermia (as in our case)[21]. The single case with 48, XXXY is also rare in such studies[22]. The case history showed our patient was born to a 33 year old father and 31 year old mother and had birthweight of 1.7 kg. He was developmentally delayed and was kept in an incubator for 28 days, suffered from frequent infections, walked at 2.5 years, and also had speech delay. At presentation the 25 year old patient was communicative, tall, and had hypertelorism, flat nasal bridge, protruding lips, prominent mandible, and radioulnar synostosis. He also had hypergonadotropic hypogonadism, hypoplastic penis, and gynecomastia.

The prevalence of Y chromosome microdeletions differs among studies from 4% in oligospermic men, to 11% in azoospermic men, to 18% in idiopathic azoospermic patients[23, 27]. Y chromosome microdeletions are affected by Y-haplotypes and Y-chromosome migration patterns[28-30]. Our results are consistent with the study of Y chromosome microdeletions in Gaza strip where no deletions were found in AZFa, AZFb or AZFc in 125 patients with primary idiopathic male infertility[19]. Similarly, such studies do not seem warranted in Israeli men who show rare cases of Y microdeletions associated with infertility[31].

Our study of 56 patients is notable for the high rate of chromosome abnormalities especially among azoospermic males. Additional studies are needed with an expanded series of studies among Palestinians to look for the causes of such a high incidence. One possibility is impact of increased pollution in the past few decades on chromosomal aberrations. Our lab actually documented a significant increase in chromosome breaks and DNA damage in Palestinians living near polluting Israeli industrial settlements[32]. However the two studies now available from Gaza[19] and our study from the West Bank suggest that chromosome studies are of paramount significant in evaluating genetic causes of infertility in Palestinian males especially those with azoospermia.

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دراسة الخلل الكروموسومي والحذف في كروموسوم Y لمجموعه من الرجال الفلسطينيين الذين يعانون من ضعف أو عدم القدرة على إنتاج الحيوانات المنوية

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الملخص

تمثل الأسباب الجينية لعقم الرجال في البلاد الغربية ما نسبته 15% من الأسباب الكلية للعقم. تعد متلازمة كلاينفلتر والحذف في كروموسوم Y في منطقه AZF من أكثر الأسباب الجينية المرتبطة بعقم الرجال الذين ليست لديهم حيوانات منوية أو عدد قليل من الحيوانات المنوية. الهدف من هذه الدراسة هو تقييم ودراسة الخلل الكروموسومي والحذف في كروموسوم Y لدى مجموعه من الرجال الذين لديهم ضعف أو عدم قدرة على إنتاج الحيوانات المنوية وهم موجودون في الجزء الجنوبي من الضفة الغربية (الأراضي الفلسطينية المحتلة). تم تحليل الخلل الكروموسومي ل 56 مريضاً بوساطة الطريقة المعيارية لدراسة الكرموسومات، وتمت دراسة الحذف في كروموسوم Y لدى 26 مريضاً بوساطة التحليل الجزيئي.

أظهرت النتائج أن وجود الخلل الكروموسومي كان مقتصراً على الذكور غير القادرين على إنتاج الحيوانات المنوية من ضمنهم 14 مريضاً يعانون من خلل في الجينات الوراثية 25٪ من جميع المرضى 37.8٪ من المرضى غير القادرين على إنتاج حيوانات منوية)، 11 مريضاً عندهم XXY 47, ومريض لكل من الحالات الوراثية غير الطبيعية XXXY47 ,48,XY, 45,X/46,XY, XXY/46,XY.

من خلال هذه الدراسة، يبدو أن لدينا نسبة مرتفعة من الخلل الكروموسومي لدى الرجال الذين يعانون من العقم مقارنه بالمجتمعات الغربية. وأنه لا أحد من المرضى كان يعاني من حذف في كروموسوم Y في منطقة AZF. وفي النطاق ذاته، أظهرت دراسة أجريت في منطقة غزه خلو مجتمعنا الفلسطيني من الخلل الناتج عن الحذف في كروموسوم Y.

استخلصت الدراسة الحالية حاجتنا الماسة إلى تحليل الخلل الكروموسومي وتشخيصه لدى الرجال الذين يعانون من عدم القدرة على إنتاج الحيوانات المنوية، وتقديم المشورة الوراثية اللازمة لهم بالرغم من محدودية الحالات التي تعاني من حذف في كروموسوم Y في مجتمعنا الفلسطيني.

الكلمات الدالة: العقم عند الرجال، الأراضي الفلسطينية المحتلة، الخلل الكروموسومي.

