Al-Azhar Med. J. *Vol. 46(3), July, 2017, 595-605*
DOI: 10.12816/0040348

CHROMOSOMAL ABNORMALITIES IN SEVERE OLIGOZOOSPERMIC INFERTILE MALES

By

Ali Ramadan Rabie[1], Ahmad Kamel Seddeik Abdel-Hameed[1,2], Mahmoud Fawzy Abdel Hamid[3], Hesham Fayek Ismail Kayed[4] and Ahmed Mohamed Maher Abdel Rahman[5]

[1]Department of Dermatology, Venereology and Andrology, Al-Azhar University Faculty of Medicine; [2]Assisted reproduction unit, the International Islamic Centre for Population Studies and Research (IICPSR), Al-Azhar University; [3]Department of Dermatology, Venereology, [4]Department of Human Cytogenetics, National Research Centre; [5]Misr University for Science and Technology

ABSTRACT

Background: Genetic abnormalities account for 15-30% of infertility in males.Chromosomal abnormalities alone are responsible for approximately 5% of male infertility.

Objective: Determining the prevalence and type of chromosomal abnormalities in a group of infertile Egyptian males with severe oligozoospermia.

Patients and methods: Karyotyping was performed on peripheral blood lymphocyte samples of 60 infertile Egyptian men with severe oligozoospermia (sperm count $\leq 5\times10^6$/ml)during the period between April 2015 and June 2016.

Results: Out of the 60 patients studied, chromosomal abnormalities were identified in 5 (8.33%) patients. All the identified abnormalities were structural autosomal abnormalities.

Conclusion: These results highlighted the importance of genetic screening and counselling for males with severe oligozoospermia before scheduling them for any of the assisted reproduction techniques.

INTRODUCTION

According to the International Committee for Monitoring Assisted Reproductive Technology (ICMART), and World Health Organization (WHO), infertility is a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after12 months or more of regular unprotected sexual intercourse **(Gurunath et al., 2011)**. It is a global problem affecting about 15% of couples worldwide**(Jungwirth et al., 2012)**. Male factor alone is responsible for 20-30% of infertile couples and contributes to another 30-40% of cases. Overall, genetic abnormalities account for 15-30% of infertility in males. Chromosomal abnormalities alone are responsible for approximately 5% of male infertility **(O'Flynn O'Brien et al., 2010)**. Many reports throughout the world showed increased prevalence of chromosomal abnormalities, structural and numerical, in males with oligozoospermia or azoospermia, and that the prevalence is higher in oligozoospermia compared to azoospermia **(Balkan et al., 2008; Kosar et al., 2010; Mafra et al., 2011; Fu et al., 2012; Ghorbel et al., 2012 and Pylyp et al., 2013)**.

With the introduction of intracytoplasmic sperm injection (ICSI), it became possible for men with severe oligozoospermia to father children of their own. However, there is a concern of probable transmission of the chromosomal abnormalities, if found, to the offspring **(Dul et al., 2012)**.

In spite of the recommendations to do karyotyping in all males with non-obstructive azoospermia or a sperm count less than 5 million per ml, this step may be bypassed for patients with severe oligozoospermia intended for ICSI.

Previous reports in Egypt **(Nazmy, 2008; Mekkawy et al., 2012 and Younan et al, 2015)** addressed the problem in both partners (males and females) and in azoospermic as well as oligozoospermic patients. No previous studies in Egypt specifically studied the chromosomal abnormalities in severe oligozoospermic patients which are more likely to bypass the genetic screening before implementation of ICSI.

The aim of the current study was to determine the prevalence and type of chromosomal abnormalities in a group of infertile Egyptian males with severe oligozoospermia.

PATIENTS AND METHODS

Sixty Egyptian men suffering from primary infertility with severe oligozoospermia (sperm count $\leq 5\times10^6$/ml) were enrolled into the current study. Patients were recruited consecutively from the attendants of the andrology clinic, assisted reproduction unit of the International Islamic Centre for Population Studies and Research (IICPSR), Al-Azhar University (Cairo, Egypt) during the period between April 2015 and June 2016. All patients underwent thorough history taking, complete physical examination, semen analysis according to the 2010 laboratory manual of the World Health Organization recommendations**(Akgul et al., 2009)**, hormonal profile assessment including FSH, LH and total testosterone (T) and karyotyping. Patients with any possible cause of poor semen parameters such as varicocele, previous exposure to gonadotoxins or obstruction were excluded from the study. The study protocol was approved by the research ethics committee of the Faculty of medicine, Al-Azhar University. The nature of the study was explained to all participants who gave an informed consent.

Chromosomal analysis was performed on peripheral lymphocyte cultures using standard methods. At least 50banded metaphase spreads were counted, 25 analysed and 12karyotyped using the imaging system.

Statistical analysis: Descriptive statistics were used to describe patients' characteristics which were presented as mean ± SD. Student's t-test was used to compare between patients with normal and abnormal karyotype results.

RESULTS

Sixty infertile men with severe oligozoospermia (sperm count $\leq 5\times10^6$/ml) were studied. Patients aged between 25 and 50 years (mean ± SD = 35.08 ± 7.64 year). Duration of infertility, sperm count and hormonal profile of all patients were summarized in table (1).

Chromosomal abnormalities were identified in 5 out of the 60 patients

(8.33%). All the identified abnormalities were structural autosomal abnormalities including 1 Robertsonian translocation and 4 reciprocal translocations (Table 2 and Fig 1-5).

There was no statistically-significant difference between patients with normal and abnormal karyotype results regarding age, duration of infertility, and levels of FSH, LH and Testosterone (T). However, there was a statistically-significant difference (P = 0.04) between the two groups regarding sperm count (Table 3).

Table (1): Age, duration of infertility, sperm count and hormonal profile of the study population. Data are presented as range (mean ± SD)..

Patient characteristic	**Range (mean ± SD)**
Age (years)	25 – 50 (35.08 ± 7.64)
Duration of infertility (years)	1 – 15 (4.74 ± 3.14)
Sperm count ($\times 10^6$/ml)	0.1 – 5 (2.11 ± 1.53)
FSH (mIU/ml)	2.4 – 20.9 (10.45 ± 5.68)
LH (mIU/ml)	2 – 9.8 (4.85 ± 2.24)
Testosterone (T) (ng/ml)	1 – 8.8 (4.63 ± 2.29)

Table (2): Chromosomal abnormalities in the studied group.

Patient's number	**The abnormal karyotype**
13	45,XY;der(13;14)(q10;q10)
29	46,XY,t(1;3)(q21;q23)
48	46,XY,der(14)t(4;14)(q10;q10)
52	46,XY,t(5;16)(q31;q24)
59	46,XY,t(9;13)(q34;q14)

Table (3): Comparison between patients with normal and abnormal karyotype results using student's t-test. Data are presented as range (mean ± SD).

Patient characteristic	**Normal karyotype**	**Abnormal karyotype**	**P value**
Age (years)	25 – 50 (35.05 ± 7.57)	27 – 50 (35.2 ± 9.12)	0.97
Duration of infertility (years)	1 – 15 (4.77 ± 3.12)	1.5 – 10 (4.4 ± 3.63)	0.83
Sperm count ($\times 10^6$/ml)	0.1 – 5 (2.3 ± 1.72)	0.1 – 2.73 (1.06 ± 1.01)	**0.04***
FSH (mIU/ml)	2.4 – 20.9 (10.57 ± 5.78)	4.2 – 15.2 (9.02 ± 4.64)	0.52
LH (mIU/ml)	2 – 9.8 (4.95 ± 2.26)	2.1 – 6.2 (3.78 ± 1.85)	0.24
Testosterone (T) (ng/ml)	1 – 8.8 (4.71 ± 2.33)	1 – 5.4 (3.74 ± 1.78)	0.3

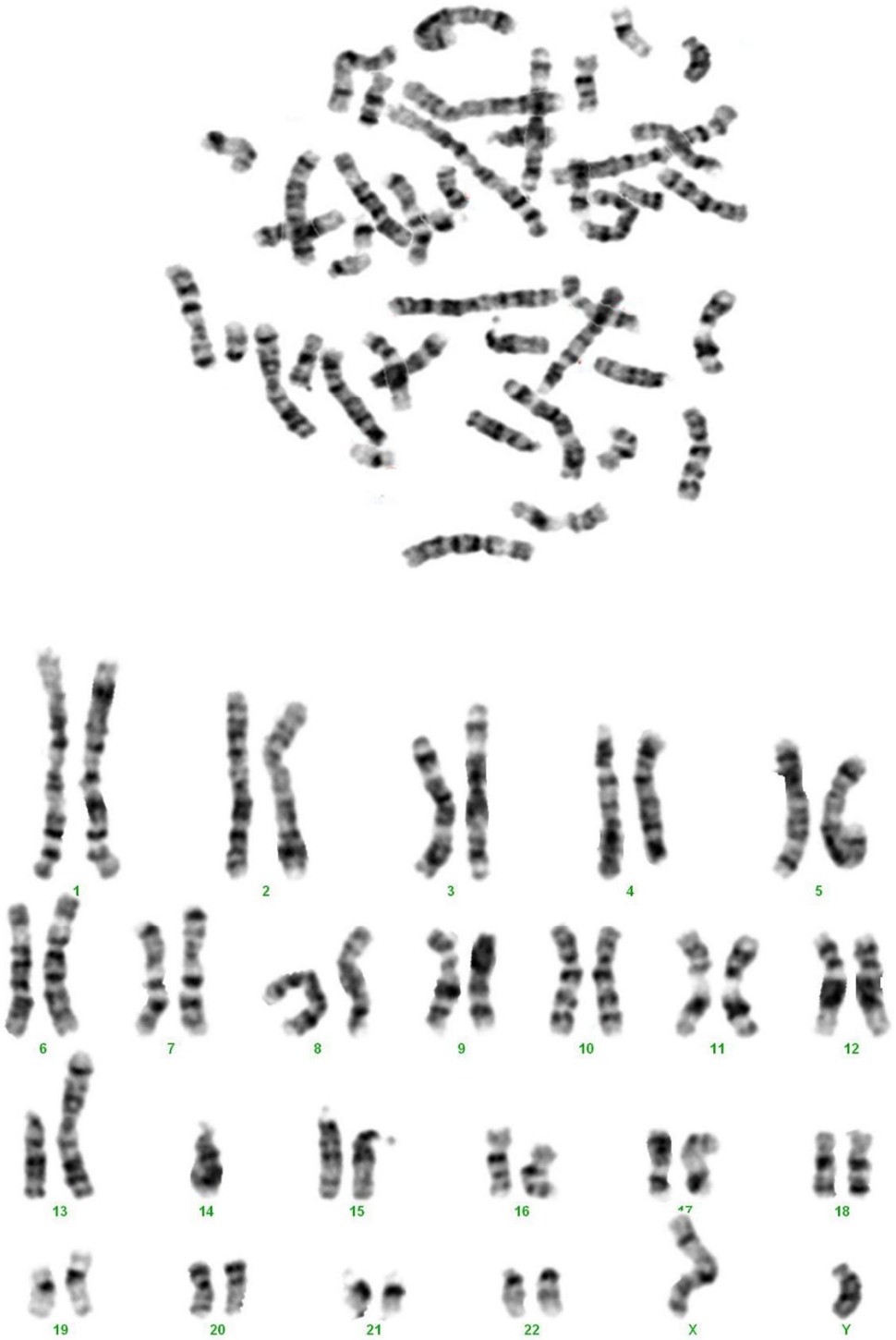

Figure (1): Karyotyping in patient no. (13) showing 45,XY;der(13;14)(q10;q10).

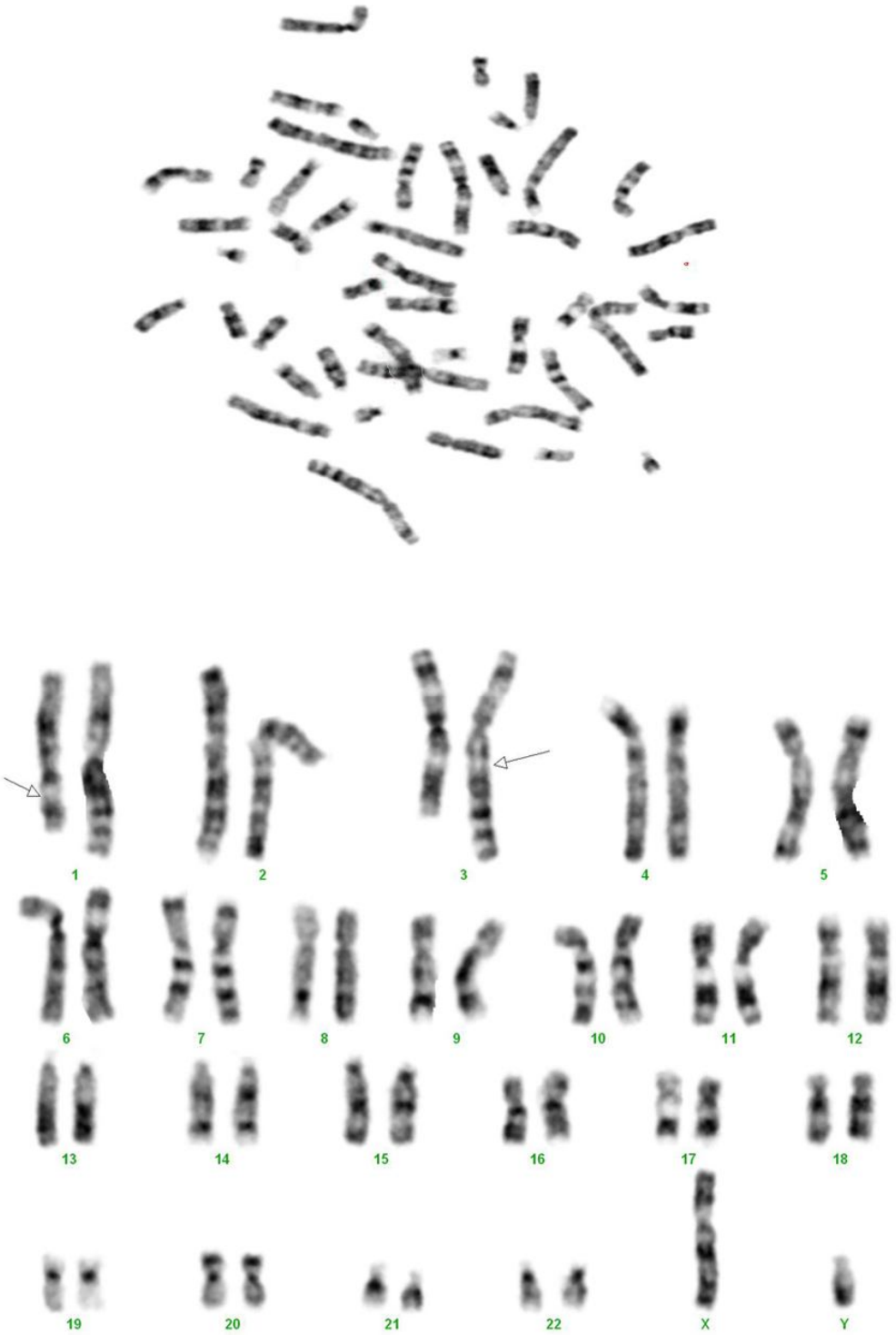

Figure (2): Karyotyping in patient no. (29) showing 46,XY,t(1;3)(q21;q23).

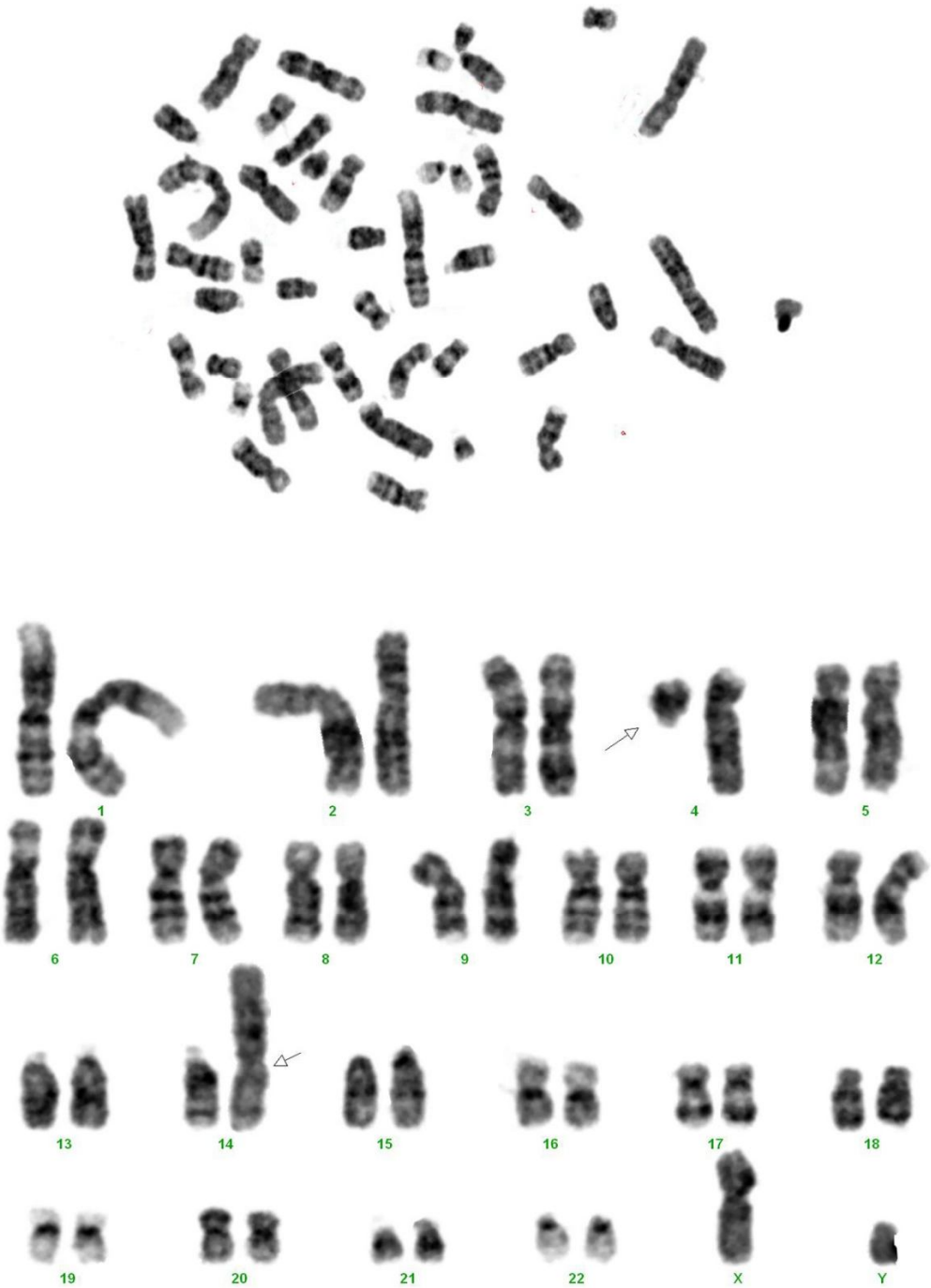

Figure (3): Karyotyping in patient no. (48) showing 46,XY,der(14)t(4;14)(q10;q10).

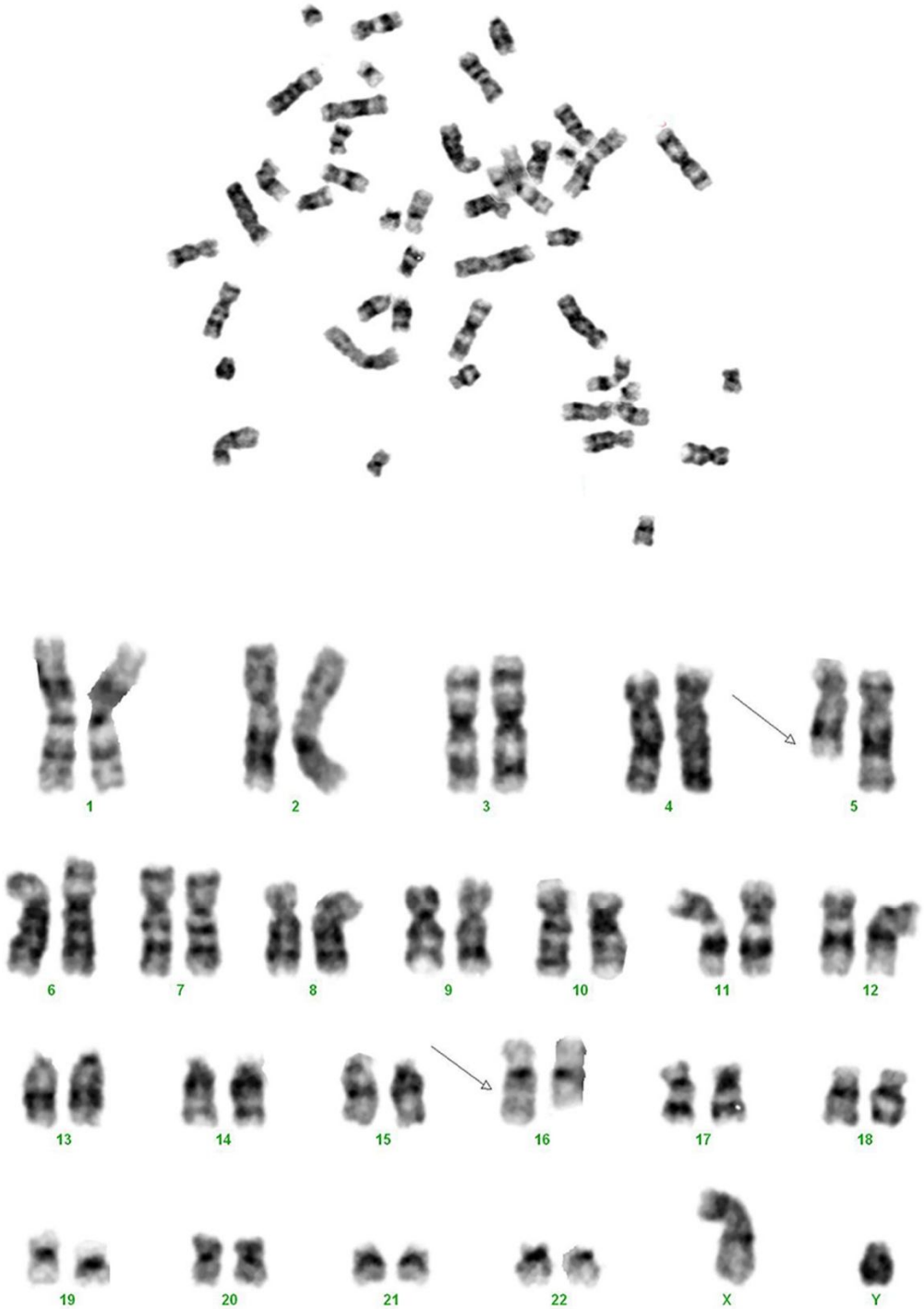

Figure (4): Karyotyping in patient no. (52) showing 46,XY,t(5;16)(q31;q24).

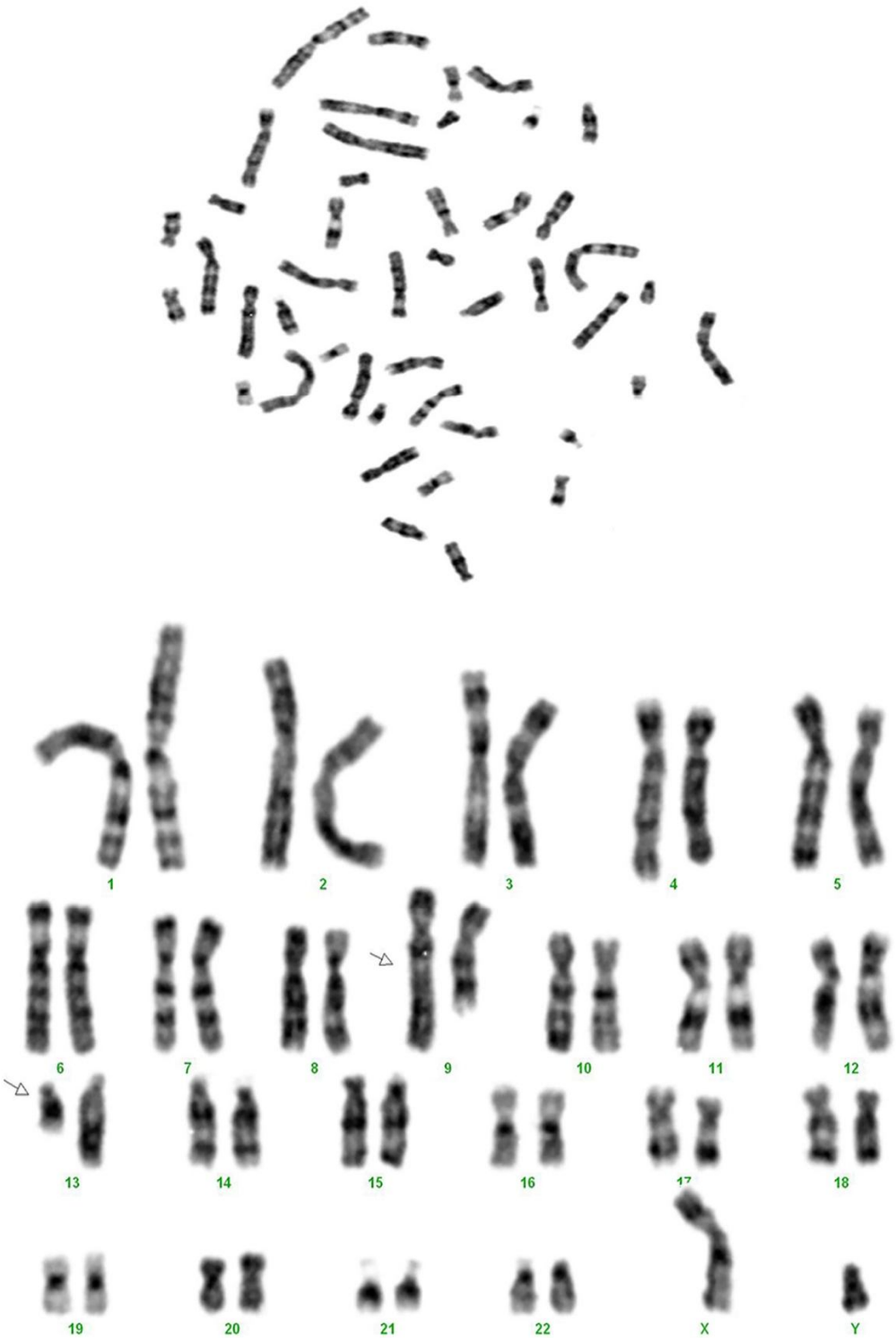

Figure (5): Karyotyping in patient no. (59) showing 46,XY,t(9;13)(q34;q14).

DISCUSSION

Among the diverse causes of poor semen parameters and male infertility, genetic defects play a significant role**(Zorrilla and Yatsenko, 2013)**. Spermatogenesis is controlled by a number of genes located on Y chromosome and autosomes as well as in the mitochondrial DNA. The products of these genes function at different stages of spermatogenesis **(Han et al., 2013)**.

In the present study, autosomal chromosomal anomalies were detected in 8.33% (5/60) of patients. Reports from different parts of the world showed variable prevalences of chromosomal abnormalities in (severe) oligozoospermia patients ranging from 0% to 18.4%**(Balkan et al., 2008; Martinez-Garza et al., 2008; Akgul et al., 2009; Kosar et al., 2010; Han et al., 2013; Pylyp et al., 2013)**. These variations could be attributed to the different genetic background of the studied populations and the severity of the oligozoospermia.

In the current study, all the anomalies detected were balanced reciprocal translocations and no sex chromosome anomalies were detected.

The absence of sex chromosome abnormalities is compatible with previous observations that abnormalities in sex chromosomes are mainly associated with severe spermatogenic failure and azoospermia, while balanced autosomal anomalies usually result in oligozoospermia **(Kumtepe et al., 2009)**.

Balanced autosomal translocations may cause oligozoospermia through interference with normal chromosome pairing and segregation at meiosis I or through dysregulation of potential genes involved in male gametogenesis at chromosome breakpoints **(Al-Achkar et al., 2013 and Alhalabi et al., 2013)**.

Males with severe oligozoospermia are now able to father children of their own using ICSI but they may pass on the chromosomal abnormalities, if present, to their offspring. It is, therefore, important to do genetic studies for those patients before ICSI procedures. However, the availability of sperms in this group of patients (unlike azoospermic patients) may encourage some to bypass this step and go directly for ICSI.

In conclusion, the results of the current study showed that chromosomal anomalies are common in severely oligozoospermic patients and they should undergo genetic screening and counselling prior to ICSI procedures.

REFERENCES

1. **Akgul, M., Ozkinay, F., Ercal, D., Cogulu, O., Dogan, O., Altay, B., Tavmergen, E., Gunduz, C. and Ozkinay, C. (2009):** Cytogenetic abnormalities in 179 cases with male infertility in Western Region of Turkey: report and review. Journal of Assisted Reproduction and Genetics, 26: 119-122.

2. **Al-Achkar, W., Wafa, A. and Moassass, F. (2013):** Cytogenetic abnormalities and Y-chromosome microdeletions in infertile Syrian males. Biomedical Reports, 1: 275-279.

3. **Alhalabi, M., Kenj, M., Monem, F., Mahayri, Z., Alchamat, G.A. and Madania, A. (2013):** High prevalence of genetic abnormalities in Middle Eastern patients with idiopathic non-obstructive azoospermia. Journal of Assisted Reproduction and Genetics, 30: 799-805.

4. **Balkan, M., Tekes, S. and Gedik, A. (2008):** Cytogenetic and Y chromosome microdeletion screening studies in infertile males with Oligozoospermia and Azoospermia in Southeast Turkey. Journal of Assisted Reproduction and Genetics, 25: 559-565.

5. Dul, E.C., Groen, H., van Ravenswaaij-Arts, C.M., Dijkhuizen, T., van Echten-Arends, J. and Land, J.A. (2012): The prevalence of chromosomal abnormalities in subgroups of infertile men. Human Reproduction (Oxford, England), 27: 36-43.

6. Fu, L., Xiong, D.K., Ding, X.P., Li, C., Zhang, L.Y., Ding, M., Nie, S.S. and Quan, Q. (2012): Genetic screening for chromosomal abnormalities and Y chromosome microdeletions in Chinese infertile men. Journal of Assisted Reproduction and Genetics, 29: 521-527.

7. Ghorbel, M., Gargouri Baklouti, S., Ben Abdallah, F., Zribi, N., Cherif, M., Keskes, R., Chakroun, N., Sellami, A., Belguith, N., Kamoun, H., Fakhfakh, F. and Ammar-Keskes, L. (2012): Chromosomal defects in infertile men with poor semen quality. Journal of Assisted Reproduction and Genetics, 29: 451-456.

8. Gurunath, S., Pandian, Z., Anderson, R. A.and Bhattacharya, S. (2011): Defining infertility - a systematic review of prevalence studies. Human Reproduction Update, 17(5): 575-588.

9. Han, T.T., Ran, J., Ding, X.P., Li, L.J., Zhang, L.Y., Zhang, Y.P., Nie, S.S. and Chen, L. (2013): Cytogenetic and molecular analysis of infertile Chinese men: karyotypic abnormalities, Y-chromosome microdeletions, and CAG and GGN repeat polymorphisms in the androgen receptor gene. Genetics and Molecular Research, 12: 2215-2226.

10. Jungwirth, A., Giwercman, A., Tournaye, H., Diemer, T., Kopa, Z., Dohle, G.and Krausz, C.(2012): European Association of Urology guidelines on Male Infertility: the 2012 update. European Urology, 62(2): 324-332.

11. Kosar, P.A., Ozcelik, N. and Kosar, A. (2010): Cytogenetic abnormalities detected in patients with non-obstructive azoospermia and severe oligozoospermia. Journal of Assisted Reproduction and Genetics, 27: 17-21.

12. Kumtepe, Y., Beyazyurek, C., Cinar, C., Ozbey, I., Ozkan, S., Cetinkaya, K., Karlikaya, G., Karagozoglu, H. and Kahraman, S. (2009): A genetic survey of 1935 Turkish men with severe male factor infertility. Reproductive Biomedicine Online, 18: 465-474.

13. Mafra, F.A., Christofolini, D.M., Bianco, B., Gava, M.M., Glina, S., Belangero, S.I. and Barbosa, C.P. (2011): Chromosomal and molecular abnormalities in a group of Brazilian infertile men with severe oligozoospermia or non-obstructive azoospermia attending an infertility service. International Brazilian Journal ofUrology: Official Journal of the Brazilian Society of Urology, 37: 244-250.

14. Martinez-Garza, S.G., Gallegos-Rivas, M.C., Vargas-Maciel, M., Rubio-Rubio, J.M., de Los Monteros-Rodriguez, M.E., Gonzalez-Ortega, C., Cancino-Villarreal, P., de Lara, L.G. and Gutierrez-Gutierrez, A.M. (2008): Genetic screening in infertile Mexican men: chromosomal abnormalities, Y chromosome deletions, and androgen receptor CAG repeat length. Journal of Andrology, 29: 654-660.

15. Mekkawy, M., Kamel, A., El-Ruby, M., Mohamed, A., Essawi, M., Soliman, H., Dessouky, N., Shehab, M. and Mazen, I. (2012): Isodicentric Y chromosomes in Egyptian patients with disorders of sex development (DSD). American Journal of Medical Genetics, 158: 1594-1603.

16. Nazmy, N.A. (2008): Cytogenetic studies of couples with reproductive failure in Alexandria, Egypt. The Journal of the Egyptian Public Health Association, 83: 255-271.

17. O'Flynn O'Brien, K.L., Varghese, A.C. and Agarwal, A. (2010): The genetic causes of male factor infertility: a review. Fertility and Sterility, 93: 1-12.

18. Pylyp, L.Y., Spinenko, L.O., Verhoglyad, N.V. and Zukin, V.D (2013): Chromosomal abnormalities in patients with oligozoospermia and non-obstructive azoospermia. Journal of Assisted Reproduction and Genetics, 30: 729-732.

19. Younan, D., Sorour, A. and Genedy, R. (2015): Aneuploidy frequency in spermatozoa of Egyptian men with normal and abnormal semen parameters using fluorescence in situ hybridisation. Andrologia, 47: 228-235.

20. Zorrilla, M. and Yatsenko, A.N. (2013): The Genetics of Infertility: Current Status of the Field. Current Genetic Medicine Reports, 1(4): 13-27.

الإعتلالات الصبغية فى الذكور العقيمين ذوى قلة النطف الشديد

على رمضان ربيع[١] - أحمد كامل صديق عبدالحميد[١،٢] - محمود فوزى عبدالحميد[٣]
هشام فايق إسماعيل قايد[٤] - أحمد محمد ماهر عبدالرحمن[٥]

[١]قسم الأمراض الجلدية و التناسلية كلية الطب بنين جامعة الأزهر؛ [٢]وحدة الإخصاب الطبى المساعد – المركز الدولى الإسلامى للدراسات والبحوث السكانية – جامعة الأزهر؛ [٣]قسم الأمراض الجلدية والتناسلية، [٤]قسم الوراثة البشرية – المركز القومى للبحوث؛ [٥]جامعة مصر للعلوم والتكنولوجيا

خلفية البحث: تمثل الإعتلالات الوراثية ١٥-٣٠% من حالات العقم لدى الرجال ، فى حين أن الإعتلالات الصبغية وحدها تكون مسئولة عن حوالى ٥% من حالات العقم لدى الرجال.

الهدف من البحث: تحديد معدل ونوع الإعتلالات الصبغية فى مجموعة من الذكور المصريين العقيمين ذوى قلة النطف الشديد.

المرضى و طرق البحث: تم إجراء التنميط النووى على عينات خلايا الدم الليمفاوية مأخوذة من ستين من من الذكور المصريين العقيمين ذوى قلة النطف الشديد (عدد النطف < ٥ مليون / مل) خلال الفترة من إبريل ٢٠١٥ وحتى يونيو ٢٠١٦.

النتائج: من بين الستين مريض الذين تمت دراستهم تم إكتشاف الإعتلالات الصبغية فى ٥ مرضى (٨،٣٣%) و كانت كل هذه الإعتلالات من نوعية الإعتلالات التركيبية.

الخلاصة: تؤكد هذه النتائج على أهمية الفحص الوراثى و المشورة للذكور العقيمين ذوى قلة النطف الشديد قبل إدخالهم فى أى من برامج الإخصاب الطبى المساعد.