UNIVERSITI PUTRA MALAYSIA

ANALYSIS OF DELETIONS IN THE AZOOSPERMIA FACTOR REGION IN SELECTED MALAYSIAN INFERTILE MALE SUBJECTS

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

May 2012

DEDICATIONS

This thesis is dedicated to my parents, my dear wife Nora, my lovely daughter Rewan and my family, who offered me love and support throughout the duration of my study

Abstract of the thesis presented to the School of Graduate Studies of University Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Chair: Prof. Patimah Ismail, PhD

Faculty: Faculty of Medicine and Health Sciences

Infertility is defined as a delay in conception of more than one year duration when there is no use of contraception. Approximately 25% of cases of male infertility are of unknown aetiology. Genetic variations have been found to be as one of the risk factors for male infertility. Among genetic variations, deletions affecting the Azoospermia Factor (AZF) region which is located in the long arm of the Y-chromosome are the most important factors in the development of male infertility. The three types of AZF deletions (AZFa, AZFb and AZFc) have been shown to be associated with male infertility. The frequency rates of those AZF deletions vary among populations due to ethnic factors. Moreover, controversy exists in the literature about the significance

of other types of AZF deletions, namely AZFd deletions and partial AZFc deletions (gr/gr, b1/b3, b2/b3 deletions), in male infertility. Conventional multiplex PCR-gel electrophoresis is the standard screening method for AZF deletions. However, this method has a number of disadvantages as it is a labor-intensive procedure with conflicting results. This study comprised 54 infertile males and 63 fertile controls (proven fertility). The frequency of AZFa, AZFb and AZFc deletions were determined using conventional PCR. In addition, a genetic association study was done to determine the association of AZFd and partial AZFc deletions between male infertility and control subjects. In this study, we have also developed a new real-time PCR High Resolution Melt (HRM) analysis based method to screen for AZF deletions and compared with the results from conventional PCR method. The results of this study showed that, three out of 54 cases (5.55%) had AZF (a, b and c) deletions (two had AZFc and one had AZFa deletions). Four cases were found to have AZFd deletions (7.4%) with two of them were found to be associated with AZFc deletions (p=0.028). In this study, gr/gr deletions were found in six cases (11.53%) as well as in six control subjects (9.52%) and significantly differed (p=0.725). One case was found to have b2/b3 deletion (1.85%, p=0.269) and there were no b1/b3 deletions identified in this study. The new HRM analysis based-method was able to produce the same results obtained with the conventional PCR-gel electrophoresis method. In fact, the HRM based-method was highly repeatable as shown by the 95% Limits of agreement statistical analysis. The frequency of AZF (a, b and c) deletions in Malaysian infertile male subjects was found to be comparable with other populations. AZFd deletions were found to be significant in male infertility and

it may be associated with other types of AZF deletions. Partial AZFc deletions were not found to be significant in Malaysian infertile males. Moreover, the HRM analysis screening method for AZF deletions was found to be reliable, reproducible, fast, probe free, of high throughput, semi-automated with easy interpretation of the results and cost-effective method.

Abstrak tesis yang dikemukakan kepada Sekolah Pengajian Siswazah Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

ANALISIS PEMADAMAN BAHAGIAN FAKTOR AZOOSPERMIA DI KALANGAN SUBJEK LELAKI MANDUL DI MALAYSIA

Oleh

HUSSEIN A. ABD AL-HUSSEIN

Mei 2012

Pengerusi: Prof. Patimah Ismail, PhD

Fakulti: Fakulti Perubatan Dan Sains Kesihatan

Ketidaksuburan ditakrifkan sebagai kehamilan lewat untuk lebih daripada tempoh satu tahun apabila tidak menggunakan alat pencegah kehamilan. Kira-kira 25% daripada punca kes ketidaksuburan lelaki masih tidak diketahui. Salah satu faktor risiko bagi ketidaksuburan lelaki adalah variasi genetik. Antara pelbagai variasi genetik, pemadaman genetik yang mempengaruhi bahagian Faktor Azoospermia (AZF) yang terletak di lengan panjang kromosom Y, adalah faktor yang paling penting dalam perkembangan ketidaksuburan lelaki. Tiga daripada jenis pemadaman AZF (AZFa, AZFb dan AZFc) telah menunjukkan pertalian dengan ketidaksuburan

lelaki. Kadar kekerapan terhadap pemotongan AZF yang berbeza di kalangan penduduk adalah disebabkan oleh faktor etnik. Selain itu, wujudnya kontroversi dalam kajian yang lepas mengenai signifikasi jenis pemadaman AZF yang lain, iaitu pemadaman AZFd dan pemadaman sebahagian daripada bahagian AZFc (pemadaman gr / gr, b1/b3, b2/b3), dalam ketidaksuburan lelaki. Multipleks PCR-gel elektroforesis konvensional adalah satu kaedah piawai saringan untuk mengesan pemadaman AZF. Walau bagaimanapun, kaedah ini mempunyai beberapa kelemahan kerana ia adalah satu prosedur intensif dengan keputusan yang bercanggah. Kajian ini terdiri daripada 54 lelaki tidak subur dan 63 kawalan kesuburan (terbukti kesuburan). Kekerapan pemadaman AZFa, AZFb dan AZFc telah ditentukan menggunakan PCR konvensional. Di samping itu, kajian genetik telah dijalankan untuk menentukan perkaitan pemadaman AZFd dan sebahagian daripada AZFc, diantara ketidaksuburan lelaki dan subjek kawalan. Dalam kajian ini, kami juga telah menemukan analisis baru berasaskan kaedah *real-time* PCR Beresolusi Leburan Tinggi (HRM) untuk mengesan pemadaman AZF dan dibandingkan dengan keputusan daripada kaedah PCR konvensional. Keputusan kajian ini menunjukkan bahawa, tiga daripada 54 kes (5.55%) mempunyai pemadaman AZF (a, b dan c, manakala 2 kes mempunyai AZFc dan satu kes mempunyai pemadaman AZFa). Empat kes telah didapati mempunyai kaitan dengan pemadaman AZFd (7.4%) dengan dua daripadanya didapati berhubungkait dengan pemadaman AZFc ($p = 0.028$). Dalam kajian ini, penghapusan gr / gr telah ditemui dalam enam orang subjek kes (11.53%) dan juga dalam enam orang subjek kawalan (9.52%) dengan signifikasi $p = 0.725$. Satu kes telah dikenal pasti

mempunyai pemadaman b2/b3 (1.85%, p = 0.269) dan tidak terdapat sebarang pemadaman b1/b3 yang berlaku dalam kajian ini. Analisis baru berasaskan kaedah HRM adalah mampu untuk menghasilkan keputusan yang sama diperolehi dengan menggunakan kaedah PCR gel elektroforesis konvensional. Malah, kaedah berasaskan HRM adalah sangat berulang seperti yang ditunjukkan oleh 95% daripada had kesesuaian analisis statistik. Kekerapan pemadaman AZF (a, b dan c) dalam subjek ketidaksuburan lelaki Malaysia yang didapati seimbang dengan penduduk negara-negara lain. Pemadaman AZFd didapati signifikan dalam ketidaksuburan lelaki dan ia boleh dikaitkan dengan jenis pemadaman AZF yang lain. Pemadaman separa AZFc telah didapati tidak signifikasi dalam kesuburan lelaki Malaysia. Disamping itu, kaedah analisis saringan HRM untuk pemadaman AZF didapati boleh dipercayai, dikeluarkan semula, cepat, tidak menggunakan probe, daya pemprosesan yang tinggi, separa automatik dengan keputusan mudah diinterpretasikan dan juga kos efektif.

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I Certify that a Thesis Examination Committee has met on to conduct the final examination of Hussein Ali Abd Al-Hussein Almeamar on his thesis entitled **"Analysis of Azoospermia Factor Region Deletions in Malaysian Infertile Male Subjects"** in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted to any other degree at Universiti Putra Malaysia or at other institution.

HUSSEIN A. ABD AL-HUSSEIN

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