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ORIGINAL RESEARCH

Utilization of Dental Pulp DNA as Diagnostic Molecular Marker for Fertility Detection in Men

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ABSTRACT

Recent advances in DNA technology have revolutionized forensic identification procedures. Teeth dentin and pulp are rich sources of DNA material, which can be successfully extracted and it provides us with valuable information on individuals, systemic health including fertility status.

Aim of study: The aim of this study was to use DNA material extracted from human teeth pulp for detection of fertility status of men.

Materials and methods: Twenty extracted premolar teeth of systemic disease free male Saudi individuals (45 years average age) were collected; eight of them were infertile while others were fertile and were used as control group. This information was concealed until the PCR analysis was performed. The results of recorded patient information was matched with the results of the DNA analysis.

Results: Results showed that the gene (sY83) an important gene of AZFa region in Y chromosome is important for male fertility. It was later evident that the infertile patients suffered from azoospermia, and that information is completely matched with our results.

Conclusion: Using DNA extracted from dental pulp can be used successfully in determining fertility status of human which may help in an accurate personal identification specially in extreme circumstances.

Keywords: DNA, Fertility markers, Dental pulp, PCR analysis.

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INTRODUCTION

Human teeth are the hardest tissue of the human body, which resist extreme circumstances such as trauma, immersion in high concentrations of acidic materials, incineration, humidity and even decomposition, so it can preserve DNA in a good pure status, with sufficient quantity for DNA analysis specially in mass disasters as other parts of the body severely destroyed (Lijnen and Willems 2001).[1] Teeth dentin and pulp are rich sources of DNA material, which can be successfully extracted and provide us with valuable information of individuals, systemic health as fertility status (Lijnen and Willems 2001; Manjunath et al 2011).[1,2] Infertility is a problem that affects both men and women everywhere in the world. Medical statistics from the US show that approximately 15% of all couples of reproductive age are unable to conceive naturally (Fernandez et al 1991).[3] Approximately 10% of couples at child bearing ages suffered from some kind of infertility and about half of these cases are because of male factors (de Kretser and Baker 1999),[2] which recently became a hot issue in the relevant studies. The Y chromosome microdeletions are the most common genetic causes of male infertility due to spermatogenesis failure and have been reported in 2.7 to 55.5% of infertile men (Krausz et al 2000)[4] and (Simoni et al 1999)[5] The frequency of Y chromosome microdeletions increases with the severity of spermatogenesis defect (Krausz and McElreavey, 1999).[6] Microdeletions in the Y chromosome long arm (Yq) are known to represent the pathogenic mechanisms for infertile males. Three distinct non-overlapping regions designated as AZFa, AZFb and AZFc are located in interval 5 to 6 of long arm of Y chromosome and are associated with impaired spermatogenesis in humans (Vogel et al 1999).[7] The microdeletions in these AZF loci are associated with azoospermia as well as varied testes histology ranging from sertoli cell only syndrome (SCO) to hypospermatogenesis (HSG) and maturation arrest. Normal testicular histology reveals two types of cells, i.e. Leyding cell and sertoli cell, both of which are required for normal functioning. Presence of only sertoli cell in testicular histology is known as SCO syndrome and is associated with infertility. These AZF regions are putative RNA binding

proteins and so may be involved in the regulation of gene expression (Rajneesh et al 2010).[8]

Subsequent PCR-based screening studies used to amplify sequence-tagged sites (STS) spanning the Y chromosome (Foote et al 1992; Vollrath et al 1992)[9,10] facilitated the detection of small interstitial deletions (i.e. microdeletions) of Yq11 not only in azoospermic (Ma, et al 1992, Reijo et al 1995, Vogt et al 1996),[11-13] but also in severely oligozoospermic men (Reijo et al 1996, Qureshi et al 1996).[14,15] Since, these deletions were variable in both extent and location, the AZF region was divided into three nonoverlapping subregions (AZFa, AZFb and AZFc) located from the proximal to the distal part of Yq (Vogt et al 1996).[13] Recent genetic studies have shown that several genes or gene families located in AZF regions are specifically expressed in the testis and could therefore be considered as AZF candidate genes for infertility, such as the RBM (RNA-binding motif) and the DAZ (deleted in azoospermia) gene families (Pera 2000 Liow et al 2001 Foresta et al 2001)[16-18] Over the past few years, screening tests for detecting micro-deletions on the long arm of the Y chromosome have established the distribution and characteristics of the deletions among different groups of infertile male patients (Pera 2000 Liow et al 2001, Foresta et al 2001).[16-18] Data from PCR studies have demonstrated that the prevalence of Y chromosome microdeletions among men with nonobstructive idiopathic azoospermia or severe oligozoospermia ranges from 3 to 55.5%, the higher frequencies being detected in cases of severely impaired spermatogenesis (Foresta et al 2001).[18] A number of factors have been implicated in the wide variation of Y deletion frequencies reported such as patient selection criteria, experimental designs, environmental influences and ethnic variations (Maurer et al 2001, Calogero et al 1999 Tse et al 2000).[19-21] So, our study aimed for testing how far we can use DNA material extracted from human teeth pulp for detection of fertility status of men which can help in accurate personal identification for medicolegal purposes.

MATERIALS AND METHODS

Teeth Samples

Twenty extracted premolar teeth samples from systemic free Saudi individuals, (45 years average age) were collected after taking written consent for participation in the study. Medical history of the patients was recorded in their dental record files. Eight from the twenty male individuals, were infertile while others were fertile and used as controls. This information was concealed until the PCR analysis was performed. The results of recorded patient information was then matched with the results of the DNA analysis.

DNA Extraction from the Collected Teeth

Genomic DNA was extracted using Mammalian tissue Genomic DNA extraction Kit (Qiagen, USA) according to manufacturer instructions. The tooth was split longitudinally into two halves and the nerve was crushed from each side of the tooth root canal space. The nerve was then transferred into eppendorf tube containing 300 µl of the lysis buffer. The extracted DNA was detected using 1.5% agarose gel containing ethidium bromide and was then visualized using UV transilluminataor and photographed using gel documentation system (Gel-Doc, 2000).

Amplification and Detection of the Fertility Specific Genes

The chosen genes were amplified by polymerase chain reaction (PCR) using specific primers (Table 1). The PCR mixture consisted of 30 picomoles of each primer, 10 ng of chromosomal DNA, 200 µM dNTPs and 2.5 units of Taq polymerase in 50 µl of polymerase buffer. The PCR was carried out for 35 cycles of 94°C for 1 minute, 55 to 65°C for 1 min according to the type of gene, 72°C for 2 minutes and final extension step at 72°C for 10 minutes. After completion, a fraction of the PCR mixture was examined using 1.5% agarose gel in TBE buffer (pH 8.5). Electrophoresis was carried out for 20 min at 150 V and photographed using gel documentation system.

RESULTS

Determination of male fertility and infertility was achieved using specific PCR primers. Deletion in a specific genetic region was identified through the failure in PCR amplification for that region. As shown in Figures 1 and 2, there is no band amplified for theses infertile samples. The gene (sY83) is known as an important gene of AZFa region which is important for male fertility. It was later known that the patient was suffered from azoospermia, and that information is completely matched with our results.

Table 1: The primers sequence and band size of the selected fertility genes

Gene	*Forward primer (5' → 3')*	*Reverse primer (5' → 3')*	*Gene size*
sY13	GTGACACACAGACTATGCTTC	TCAAGGTTGTTGTTTAAGCT	187
sY83	CTTGAATCAAAGAAGGCCT	CAATTTGGTTTGGCTGACAT	275
sY283	CAGTGATACACTCGGACTTGTGTA	GTTATTTCAAAAGCTACACGGG	497
RPM1	CTTGAAAACAATTCCTTTTCC	TGCACTTCAGAGATACGG	800

The results shown in Figures 3 and 4, revealed that the deletion of sY283 and RPM1 genes was only observed in samples from fertile individuals. The mentioned sY283 gene considered as important gene for fertility. The gene is a part of AZFc region specially DAZ gene region which play an important role in fertility. Those individuals proved sufferring from oligozoospermia according to their medical record information.

According to SãoPedro et al 2003,[22] only the severely oligozoospermic patients failed to amplify sY283 gene, which amplify parts of the DAZ gene in the AZFc region.

DISCUSSION

The total production of genomic DNA obtained from the fresh dental sample may range from 6 to 50 μg DNA[23] and the PCR method enables differentiation of one individual from another, with a high level of reliability, and with about 1 ng (one billionth of a gram) of the target DNA.[23] Thus, abundance of quality DNA can be extracted from a tooth, which is an important advantage in DNA analysis.[24,25] In comparison to soft tissues, hard tissues are greatly resistant to autolysis and decay caused by environmental factors, which is why bones, teeth and nails are the only source of DNA in some forensic cases.[26] Polymerase chain reaction (PCR) technique that allows amplification of DNA at pre-selected sites of biological material extracted even from a root filled tooth will be sufficient to make a conclusion on the identity of a person.[27,28] The AZF regions include genes that encode proteins implicated in male spermatogenesis. Moreover, among these genes, DDX3Y (DEAD-box RNA helicase Y, formerly DBY) in AZFa, RBMY1 in AZFb and DAZ in AZFc are considered strong AZF candidates because they are frequently deleted in infertile men. Then they are exclusively expressed in human testes, and their homologues in other species have a role in spermatogenesis.[29] Another Y chromosome genes, likely implicated in spermatogenesis but not related to microdeletions, TSPY is a candidate oncogene that, due to its limited expression pattern in germ cells, is thought to function as a proliferation factor during spermatogenesis.[29,30] A study by Hossam, 2012[31] stated that, distinguishing between fertile and infertile males was 28.5% (4 out of 14) with false positive results 16.6%. (1 out of 6), false negative results 14% (2 out of 14), nonfertile 30% (6 out of 20) which was consistent with the medical history from patient's record files.[31] In most mammals, the male is identified by amplifying the SRY gene (sex-determining region Y) which is a Y chromosome-specific sequence.[32] The multiplex-PCR succeeds to differentiate between the male and female teeth, by amplified different amplicones with different molecular sizes from the genomic DNA of both. The five examined genes are distributed along the Y chromosome; sY13 from the long arm, sY83 and SY 90 from AZFa region, RPM1 from AZFb region, CDY from the DAZ region. Furthermore, recent reports have described the use of the mitochondrial DNA because the DNA on the teeth tissues was not enough, in this study the extracted of DNA from the tooth pulp was enough for all the analysis required in similar analysis. The results of this study showed the gene (sY83) which is known as an important gene of AZFa region which is important for male fertility is deleted in infertile individuals. It was later known that those patients suffered from azoospermia

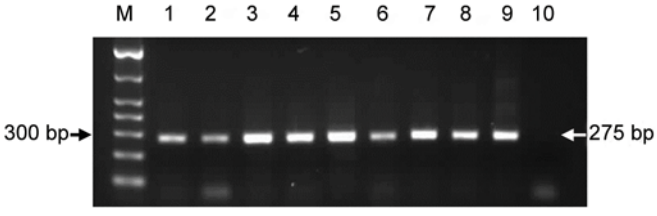

Fig. 1: PCR products of the amplified sY83 gene using specific primers, M; 1kb DNA ladder, 1-10; the patients

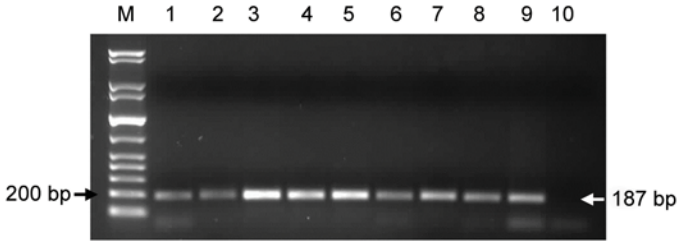

Fig. 2: PCR products of the amplified sY13 gene using specific primers, M; 1kb DNA ladder, 1-10; the patients

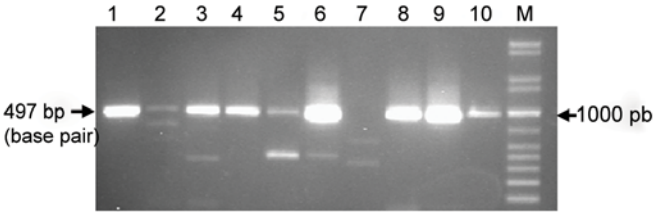

Fig. 3: PCR products of the amplified sY283 gene using specific primers, M; 1kb DNA ladder, 1-10; the patients

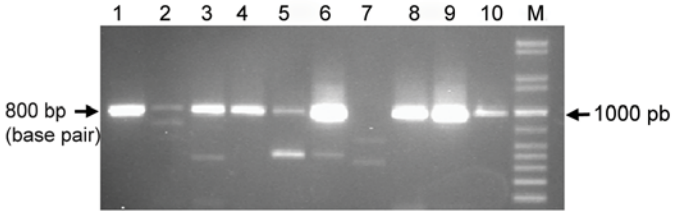

Fig. 4: PCR products of the amplified RPM1 gene using specific primers, M; 1kb DNA ladder, 1-10; the patients

according to their medical reports, and that information is completely matched with our results. Identification of fertility genes gave positive results in 75% (6 out of 8).

CONCLUSION

Using DNA extracted from dental pulp can be used successfully in determining fertility status of human which may help in an accurate personal identification specially in extreme circumstances.

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