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Original Article:

AZF Microdeletions in Human Semen Infected with Bacteria

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Abstract: Bacterial infections are associated with infertility in men. This study was aimed to investigate microdeletions on Yq chromosome in semen infected with bacteria by using bacteriological, biochemical, and serological assays. The investigation showed that 107 of 300 (84.80%) semen samples collected from infertile men with primary or secondary infertility were infected with different species of bacteria. *Chlamydia trachomatis* and *Neisseria gonorrheae* were the most frequently diagnosed bacteria in the infected semen samples. The percentages of infections of semen samples with *C. trachomatis* and *N. gonorrhea* were 42.31% and 35.28% respectively. Genomic DNA from each semen sample infected with predominant bacteria was analyzed for AZF deletions by using multiplex PCR. Different patterns of AZF microdeletions were obtained. It can be concluded that sexually transmitted bacteria may contribute in microdeletions of Yq chromosome by indirectly producing reactive oxygen species and causing gene defect in AZF regions.

Key Words: Male infertility; Yq microdeletion, *Chlamydia trachomatis*, *Neisseria gonorrheae*

Introduction:

Male infertility has been associated with a number of non-genetic and genetic factors.[1] The non-genetic factors include hypogonadotrophic hypogonadism, previous inguinal and scrotal surgery, and environmental factors such as genital infections. In respect to male urogenital tract infection, it was found that asymptomatic bacteriospermia had an important role in male infertility through affecting different sites of male reproductive tract, such as the testis, the epididymus and male accessory gland.[2]

The contribution of genetic factors in male infertility has been reported. In this respect, it was observed that Yq chromosome, and in particular Yq11.23 was involved in the sexual development and spermatogenesis.[3] Yq contains three AZF regions designated AZFa, AZFb, and AZFc from proximal to distal Yq.[4] A more recent study reported a new region designated AZFd and it was mapped between STS and sY145-sY221.[5] Many microdeletions on Yq have been implicated as significant causes of infertility. These microdeletions are often observed at Azoospermia Factor (AZF) locus.[6] AZF locus harbor genes, *RBMY* (RNA Binding Motif on Y) and *DAZ* (Deleted in Azoospermia), that involved in spermatogenic failure.[7]

Many researchers have linked positive bacterial semen cultures with poor semen quality[8-10] but to best of our knowledge no study has correlated between the existence of bacteriospermia and gene's defect on Yq chromosome. Thus, this study was aimed to investigate the occurrence of microdeletions on Yq in semen infected with bacteria.

Patients and Methods

The study was conducted during January 2010 to December 2010 on 300 infertile men attending Kamal Al-Samaraie Hospital/IVF Center in Baghdad, Iraq. The patients had either primary infertility (n=242) or secondary infertility (n=58). Those patients were interviewed about their medical history, family backgrounds, reproductive problems and possible consanguinities. In addition, a physical examination was conducted in all cases, in order to identify anatomical problems. In addition, 30 fertile men were used as control. Semen samples were obtained after a 7 days period of sex-inhibition.

The semen samples were collected from patients following the World health organization (WHO) guidelines[11], and were subjected to microbiological tests for identification bacteria. Each sample was cultured on different selective media (Blood agar, Chocolate agar, MacConkey agar, Mannitol salt agar, Chromogenic UTI agar) and incubated at 37ºC for 24-48 hrs. After incubation period, the colonies were identified by using standard bacteriological and biochemical assays.[12,13] Furthermore, several specific tests were used to detect the suspected colonies, for example BactiCard *Neisseria* test was used to detect *Neisseria gonorrheae*, H.V.D.R.L test was used to identify *Treponema pallidum*, and Furazolidone disk test was used to differentiate Staphylococci from Micrococci.[14,15] ELISA assay was also used to detect anti-*Chlamydia* trachomatis antibodies IgG by using a specific kit (Euroimmune, Germany).[16]

Genomic DNA was isolated from each seminal fluid samples infected with predominant bacteria by QIAamp®DNA Mini Kit (Qiagen, Valencia, CA). The screening for AZF microdeletions was performed by multiplex PCR. A series of 16 of specific STSs, mapped in four AZF regions, human zinc-finger protein-encoding genes (ZFX/ZFY) located on the X and Y chromosomes, acting as an internal control primers, and a unique region of *SRY* on Yp chromosome for detection XX male arising from Y to X translocation were selected [17,18]. The specific STSs included sY86 and sY84 for *AZFa*, sY127, sY134, sY121, sY124, sY128, sY130 for AZFb, sY254, sY157, sY255, sY242,

sY208 for AZFc, sY133, sY152, sY145 for AZFd. Multiplex PCR amplification was performed in a 10µl reaction system, containing 200 ng of genomic DNA, 1.5 mmol/L Mg^{2+}, 800 µmol/L deoxynucleotide triphosphates (dNTPs), 10 pmol/L of each primer and 2 U Taq polymerase. Amplification was carried out in thermocycler (Appendorf, Germany). The conditions for PCR amplifications consisted of an initial denaturation of 6 min at 94ºC, followed by 35 cycles of 40 s at 94ºC, 45s at 55ºC and 60s at 72ºC, with final extension step at 72ºC for 6 min.

Thirty fertile men were used as positive control to ensure the performance of the amplification reaction. The PCR products were separated on 1.5%agarose gel for 2hrs at 5V/cm. The results were visualized using UV transilluminator system.

Results:

Out of 300 cultured semen, 107 (84.80%) showed positive bacterial contamination (Table 1). *Chlamydia trachomatis* antibodies IgG were diagnosed in 58 (42.31%) samples. This bacterium markedly formed higher prevalence in secondary infertile men than primary infertility cases; the percentages were 24.13% and 18.18%, respectively. The second dominant bacteria was *Neisseria gonorrheae;* it was identified in 27 (11.15%) semen samples obtained from patients had primary infertility and in 14 (24.13%) cases had secondary infertility. In addition, *Treponema pallidum* was successfully isolated from 3 (1.23%) samples obtained from patients with primary infertility and 2 (3.44%) patients with secondary infertility. Whereas *Staphyloccocus aureus* was existed in lower incidence; it was found at 0.41% and 1.72% in primary and secondary infertile men respectively. Trailing behind these was *Escherichia coli* which isolated only from one patient (0.41%) with primary infertility. It is worth mentioning, no culture yielded a mixed growth of bacteria; on the other hand no bacterial isolates were recovered from samples of fertile men's semen.

Table 1: Bacteria isolated from semen samples of infertile men. (Control showed no bacterial infection)

Type of bacteria	Type of infertility	
	Primary infertility (n=242)	Secondary infertility (n=58)
	No. (%)	No.(%)
C. trachomatis	44 (18.18)	14 (24.13)
N. gonorrhea	27 (11.15)	14 (24.13)
T. pallidum	3 (1.23)	2 (3.44)
S. aureus	1 (0.41)	1 (1.72)
E. coli	1 (0.41)	0 (0)
Total	76 (31.38)	31 (53.42)

Subsequently semen samples infected with prevalent bacteria, *C. tarchomatis* and *N. gonorrheae*, were subjected to molecular analysis of Yq microdeletion. Table 2 showed the analytical results of multiplex PCR reactions concerning patients who had either primary or secondary infertility infected by *C. trachomatis*. Semen samples from primary infertile men revealed 3 (6.81%) samples with complete deletion in AZFa+b+c+d, 11(25%) samples observed with partial deletion in AZF b+d and complete deletion in AZFc, 8 (18.18%) harbored partial deletion in AZFb+c+d, and 22 (50%) showed complete deletion in AZFc. While secondary infertile men who harbored the same bacterium showed 10 (64.28%) samples had complete deletion in AZFc, 3 (21.42%) samples indicated complete deletion in AZFc and partial deletions in AZF b+d, and one (7.14%) sample partial deletion in AZFb+c+d.

Table 2:AZF deletions detected in semen infected with *C. trachomatis*

Type of bacteria	Type of infertility	Semen samples No. (%)	Deletion in AZF loci	Type of deletion
C. trachomatis	Primary infertility	3 (6.81)	a+b+c+d	complete
		11 (25)	a	*
			c	complete
			b+d	partial
		8 (18.18)	a	*
			b+c+d	partial
		22 (50)	a	*
			b	*
			c	complete
			d	*
	Secondary infertility	10 (64.28)	a	*
			b	*
			c	complete
			d	*
		3 (21.42)	a	*
			c	complete
			b+d	partial
		1 (7.14)	a	*
			b+c+d	partial

*Skipping band

On the other hand, DNA of semen from infertile patients infected by *N. gonorrheae* was analyzed for screening AZF microdeletions (Table 3). In primary infertile men, 14 (63.63%) semen samples exhibited partial deletion in AZFb+c+d, 3 (13.36%) revealed complete deletion in AZFb+c+d, 3 (13.36%) showed partial deletion in AZFb and complete deletion in AZFc, and 2 (9.09%) cases had complete deletion in AZFd. While semen specimens of secondary infertile men showed 3 (42.85%) cases had partial deletion in AZFc, 2 (28.57%) cases had partial deletion in AZFb+d, one (14.28%) sample showed complete deletion in AZFb, and one (14.28%) sample appeared with complete deletion in AZFa. Regarding semen of fertile men, no AZF microdeletions were noticed.

Table 3: AZF deletions detected in semen infected with N. gonorrhea

Type of bacteria	Type of infertility	Semen samples No. (%)	Deletion in AZF loci	Type of deletion
N. gonorrhea	Primary infertility	14 (63.63)	a	*
			b+c+d	partial
		3 (13.63)	a	*
			b+c+d	complete
		3 (13.63)	a	*
			b	partial
			c	complete
			d	*
		2 (9.09)	a+b+c	*
			d	complete
	Secondary infertility	3 (42.85)	a	*
			b	*
			c	partial
			d	*
		2 (28.57)	a	*
			b	partial
			c	*
			d	partial
		1 (14.28)	a	*
			b	complete
			c	*
			d	*
		1 (14.28)	a	complete
			b	*
			c	*
			d	*

*Skipping band

Discussion:

The occurrence of male genital tract and/or accessory gland infections has been considered as a potential hazard to male infertility. In this study, 107 (84.80%) of examined seminal fluid samples, collected from 300 patients with primary and secondary infertility, were infected with different species of bacteria: *C. trachomatis*, *N. gonorrheae*, *T. pallidum*, *S. aureus*, and *E. coli*. The obtained results showed that *C. trachomatis* and *N. gonorrheae* were the most prevalent bacteria isolated from semen; the percentage was 42.31% and 35.28%, respectively. It is worth mentioning, other investigators reported that the genital tract infections and inflammation were associated with 8-35% of male's infertility cases[8,19] and these bacteria were considered as asymptomatic bacteriospermia. Furthermore, there are indications that sexually transmitted infections, especially *N. gonorrheae* and *C. trachomatis*, account for a significant proportion of cases causing occlusion of the vas deferens and subsequent oligospermia and azoospermia.[20]

Hosseinzadeh and his group[21] reported that the active component of *C. trachomatis*, lipopolysaccharide, was responsible for sperm death due its spermicidal properties. Research work conducted by Gomez and coworkers showed that the presence of *N. gonorrheae* in semen may result in azoospermia or oligospermia, then lead to infertility by binding to human sperms, and result in agglutation of sperms and decreasing the sperm's motility.[22] Kauer and his research team[23] were able to isolate *S. aureus* (2.13%) and *E. coli* (0.14%) from infertile semen's cultures. These bacteria can be considered as commonsalty bacteria. Whereas *T. pallidum*, a bacterium that cause syphilis, was reported to be associated with male infertility by lowering the sperm count and volume of ejaculate.[24]

In this study, the possible connection between bacterial infection and potential gene defects in human semen, which infected with predominant bacteriospermia, *C. trachomatis* and *N. gonorrheae,* was investigated. Regarding semen infected with *C. trchomatis* (Table 2), the most frequently microdeletions were seen in AZFc (85.70%). It was reported that the complete deletion in AZFc region (absence of sY254, sY157, sY255, sY242, sY208) means defect in *DAZ* gene family that had been regarded as the most likely candidate genes for spermatogenesis deficiency.[17] In addition, other study reported deletion in AZFa+b+c+d regions (3 of 44 cases, 6.81%), they suggested that this region probably involves the heterochromatic region on Y chromosome and causes severoligozoospermia.[5] Whereas other study conducted by Kin (2004)[5] reported that 7.14% of cases had deletion in AZFd, he suggested that deletion in this region may not be associated with extent of defective spermatogenesis.

On the other hand, our results (Table 3) showed that the semen infected with *N. gonorrheae*, revealed 3 (13.63%) cases with primary infertility and the genome harbored deletion in AZFc. The obtained results showed that 14.28% of examined cases gave complete deletion in AZFa and AZFb. It had been reported that absence or deletion of AZFa, which harbors two genes, *USP9Y* and *DBY*, will cause spermatogenic failure, while deletion of AZFb, which contains *RBMY1*; will cause spermatogenic arrest.[5] Further studies are required to explain the mechanism which causes the deletion.

In general, when microbes invade the human body, it produces polymorphonuclear leukocytes and macrophages, which are the major sources of reactive oxygen species (ROS) production.[27] Although there is no direct evidence that *N. gonorrheae* or *C. trachomatis* increases ROS production, the associated leukocytospermia is well known to produce ROS.[28] Most importantly, leukocytospermia has been associated with occult sperm DNA damage, this may occur directly in the form of leukocytospermia, a manifestation of inflammation that is associated with cytokines, which can potentially alter spermatogenesis and cause DNA aberrations, or indirectly as a result of pathological ROS levels, which are frequent in leukocytospermia patients.[29]

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