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Shorter leukocyte telomere length is associated with risk of nonobstructive azoospermia

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Objective: To determine the association between leukocyte telomere length and the risk of nonobstructive azoospermia (NOA).
Design: The mean leukocyte telomere length (LTL) among men with NOA, obstructive azoospermia (OA), and normospermic subjects was determined by quantitative polymerase chain reaction (PCR). We used logistic regression to investigate the association between LTL and the risk of NOA after adjustment for age and body mass index (BMI). Partial correlation analysis was also used to evaluate the relationship of clinical parameters with the mean LTL among men with OA and NOA.
Setting: Reproductive medicine center.
Patients(s): A total of 866 men, including 270 normospermic controls, 247 OA and 349 NOA patients.
Intervention(s): None.
Main Outcome Measure(s): Leukocyte telomere length.
Result(s): The mean relative LTL of men with NOA was significantly shorter than that of those with OA and in normospermic controls (odds ratio [OR] 0.81, 95% confidence interval [CI] 0.64–0.98 vs. OR 0.92, 95% CI 0.70–1.24 vs. OR 0.99, 95% CI 0.83–1.22), respectively). Subjects with shorter telomeres (lowest tertile) had a significantly higher risk of NOA than those with longer telomeres (highest tertile). Interestingly, we also found that a low relative LTL was associated with poor efficiency of spermatogenesis using the Johnsen score after testis biopsy and histopathology in azoospermic patients, after adjusting for patient age and BMI.
Conclusion(s): This is the first report that short LTL is associated with NOA, shedding light on an important biological pathway involved in the etiology of this form of male factor infertility. (Fertil Steril® 2018;110:648-54. ©2018 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: Leukocyte telomere length, azoospermia, infertility

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Infertility affects 10%–15% of married couples worldwide (1). Approximately half of all cases involve male factors, and 10%–15% of these entail azoospermia (2). Azoospermia comprises obstructive azoospermia (OA) and nonobstructive azoospermia (NOA). The OA, which comprises 40% of such cases, is the consequence of physical blockage to the male excurrent duct system, and is typically accompanied by preservation of normal exocrine and endocrine functions, and normal spermatogenesis (3). The NOA, which accounts for approximately 60% of patients with azoospermia, is a complex, multigenetic disorder with a high degree of heritability that is caused by genetic and environmental factors (4–6). Several genetic factors linked to NOA have been reported, including whole chromosomal aneuploidy, Y chromosome microdeletions, and autosomal mutations (7–10). However, the etiology of most (~75%) cases of spermatogenic failure in humans remains unknown (11, 12).

Telomeres are evolutionarily conserved hexametric tandem repeats and are located at the ends of eukaryotic chromosomes, capable of preventing end degradation and maintaining genomic integrity (13). The roles of telomeres and their length in reproduction have been studied previously. Thus, defective synapsis and reduced

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recombination between homologous chromosome were found in telomerase null ($TR^{-/-}$) mouse strains, resulting in poor embryo development (14, 15). We also demonstrated that sperm telomere length was significantly associated with human embryonic development (16). In recent years, many studies have been published on sperm telomere length in relation to semen parameters. Some studies (16–18) reported that it was positively associated with sperm counts in semen. Another study (19) found that the sperm telomere length was negatively associated with sperm aneuploidy and DNA fragmentation. All of these studies showed that sperm telomere length was strongly associated with semen quality. In addition, another study (20) reported that the sperm telomere length of idiopathic infertile men was significantly lower than that of controls, and other studies (18, 21) showed that sperm telomere length and leukocyte telomere length (LTL) tended to be correlated in the same individual.

Therefore, we hypothesized that LTL might play an important role in the pathophysiology of azoospermia. However, no studies have reported this association. Hence, the aim of this study was to analyze the mean LTL in men with NOA and OA and in normospermic control subjects, and to correlate it with clinical parameters in azoospermic patients.

MATERIALS AND METHODS

Patient Population

Standard semen analysis was performed three times after 3–5 days of sexual abstinence. Azoospermia was defined as the absence of spermatozoa in centrifuged specimens. These patients were further classified as OA or NOA by evaluating their medical history, physical examination, serum hormone levels (FSH, LH, T, PRL, E_2, and P), karyotyping, Y chromosome microdeletion analysis, and imaging studies, percutaneous epididymal sperm aspiration (PESA) or testicular sperm aspiration (TESA). The OA was defined by physical obstruction in the male reproductive system. These patients usually have normal testicular volume, hormone levels, and indurated epididymides. Nonobstructive azoospermia was defined by spermatogenic dysfunction, and these patients usually have abnormal hormone levels and small, soft testes (22, 23).

In the present retrospective study, 866 subjects (270 normal men, 247 patients with OA and 349 with NOA) were recruited from the reproductive medical center of the First Affiliated Hospital of Zhengzhou University from September 2013 to October 2015. The 349 NOA men in the present study had testicular or epididymis biopsy. There were 51 men with Sertoli cell-only syndrome and 57 had spermatogenic arrest. These men were identified based on the testis biopsy and histopathology diagnosis by hematoxylin-eosin (H & E) staining. All patients had normal karyotypes with no Y chromosome microdeletions. Normospermic controls with sperm counts $\geq 39 \times 10^6$/mL. according to a previous study (18), were recruited from among couples with known female factor infertility, such as ovulation failure, fallopian tube blockage, endometriosis, or uterine malformation. Men with a history of varicocele, mumps, epididymitis, cryptorchidism, orchitis, using chemotherapy drugs, or having undergone radiation treatment were excluded. Testicular volumes were measured using a Prader orchidometer. Serum hormone levels were measured using electrochemiluminescence immunoassays. The Johnsen score (24) was used to analyze the histopathological characteristics of testicular biopsies recovered by TESA. Body mass index (BMI) was recorded as mass divided by height squared in kilograms per meter squared. This study was conducted with the approval of the Institutional Review Board of the First Affiliated Hospital of Zhengzhou University.

LTL Assay

Genomic DNA was extracted from peripheral blood leukocytes using a DNA Mini Kit (Qiagen #51106) according to the manufacturer's recommendations. The LTL was then determined using an established and validated quantitative real-time polymerase chain reaction (PCR)-based method, as reported in our previous study, with some modifications (25, 26). Briefly, relative telomere length was calculated as the telomere/single copy gene ratio, which represents the average telomere length of the sample because amplification is proportional to the number of primer-binding sites in the first PCR cycle (27). All samples were run on 96-well plates using a 7500 Real-Time PCR system (Applied Biosystems). The ratio of the telomere repeat copy number and the single-copy gene *36B4* (a single copy gene located on chromosome 12) were assayed as separate reactions on separate plates. Each sample was run in triplicate and each PCR run was carried out using 10 ng DNA in a 20-μL final reaction volume. A control sample was added to each plate to reduce interassay variability. The amplification efficiency was calculated for each plate based on a standard curve prepared using a same patient genomic DNA. Mean threshold quantification (Ct) values were used to compute the relative telomere length using the telomere/single copy gene ratio according to the following formulae: $\Delta Ct_{sample} = Ct_{telomere} - Ct_{control}$; $\Delta\Delta Ct = \Delta Ct_{sample} - \Delta Ct_{reference\ curve} = (Ct_{sample} - Ct_{control}) - (Ct_{sample} - Ct_{control})$; and then telomere/single copy gene $= 2^{-\Delta\Delta Ct}$. The mean correlation coefficients of telomere repeat copy number and single-copy gene *36B4* standard curves were 0.979 (range, 0.951–0.998) and 0.982 (0.960–0.992), respectively. The efficiency values of the telomere repeat copy number and single-copy gene *36B4* estimates were 104.1% (range, 100.8%–109.7%) and 105.1% (100.6%–109.7%), respectively. The mean coefficient of variation of telomere/single copy gene ratios was 3.24% (range, 1.54%–3.69%).

Statistical Analyses

Normal distribution of the data was examined by the Shapiro-Wilk test. Data are reported as median (25–75th percentile). Characteristics of the OA and NOA cases and normal subjects were compared using the Kruskal-Wallis H test. The correlations between telomere length and quantitative variables were analyzed by calculating partial correlations. The distributions of relative telomere length were divided into three

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