Renalase Gene rs2576178 Polymorphism in Hemodialysis Patients: Study in Bosnia and Herzegovina

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ABSTRACT

Introduction: Renalase is a protein secreted in kidneys and considered as a blood pressure modulator. High rates of hypertension and its regulation in patients on hemodialysis demands search for potential cause and treatment. The aim of this study was to determine the genotype and allele frequencies of renalase gene rs2576178 polymorphism in population from Bosnia and Herzegovina. Also, the objective of present study was to find the possible association between renalase gene rs2576178 polymorphism and hypertension in patients on hemodialysis. Material and Methods: The genotype of renalase gene rs2576178 polymorphism was determined in 137 participants (100 patients on hemodialysis and 37 controls), using polymerase chain reaction (PCR) and subsequent cleavage with MspI restriction endonuclease. Genotype and allele frequencies were assessed for Hardy-Weinberg equilibrium using a Chi-squared test. The value of P<0.05 was considered as statistically significant. Results: Comparison of genotype distribution and allele frequency in participants on hemodialysis with and without hypertension, and healthy control showed no statistical difference. Conclusion: The results of the study suggest that renalase gene rs2576178 polymorphism is not a factor that influences blood pressure in patients on hemodialysis. Key words: renalase gene, hypertension.

1. INTRODUCTION

Renalase was discovered in 2005 by groups of scientists guided by Xu and Desir and proposed to be blood pressure and cardiac function regulators (1). It is believed that Renalase is a hormone, mainly synthesized by kidneys and excreted into the blood, but recently, there were different findings. First report about this flavin adenine dinucleotide (FAD)-dependent protein in different region in Central Nervous System and peripheral nerves was published 2010, revealing new insight in its role (2).

Renalase was classified as a flavoprotein that functions as a FAD/NADH oxidase and metabolizes circulating catecholamines (1). So, it may play the role in the regulation of sympathetic tone and modulates blood pressure and cardiac function. Possible, it regulates disposition of neurotransmitter in the brain.

Renalase gene is located on chromosome 10 (q23,33) and encodes 331 kbp long protein that belongs to flavin adenine dinucleotide dependent amine oxidase.

The gene has 10 exones. There are several isoforms of renalase but the major one contains 342 amino acids with signal peptide, FAD binding domain, and monoamine oxidase domain (3).

There is a single nucleotide polymorphism at the 5' flanking region rs2576178 GG that has been linked to hypertension (4).
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| Renalase polymorphism | Sequence of primers | Restriction enzymes | AA genotype | GG genotype |
|-----------------------|---------------------|---------------------|-------------|------------|
| rs 2576178            | Sense: 5'AGTGCCGTTCAACAGTTAG 3' | MspI | 200 bp | 160 + 40 bp |
|                       | antisense: 5'GTGGGCTATTGTGGAGAA3' |        |          |            |

Table 1. Primers sequence and base pairs for renalase polymorphism genotypes

In advanced stages of the chronic kidney disease—end stage renal disease (ESRD), hypertension is presented in more than 80% of the patients, reaching up to 90% in those treated with hemodialysis (5). Hypertension is considered as a major risk for various cardiovascular diseases that are responsible for high mortality rate in patients on hemodialysis. Sympathetic nervous system function is associated with chronic renal failure (6). Besides the reduced catecholamine clearance, increased sympathetic nerve activity may be one of the causes of the increased plasma catecholamine levels evident in ESRD patients.

There isn’t clear association between blood pressure values and mortality in ESRD patients, but it is believed that regulation of hypertension should be beneficial to the ESRD patient. Concerning the fact that the etiology of high blood pressure is multifactorial, especially in patients on hemodialysis, we investigated possible role of the renalse gene rs2576178 polymorphism in pathophysiology of hypertension in patients on hemodialysis. Also, objective of study was to determine the genotype and allele frequencies of renalse gene polymorphism in Bosnia and Herzegovina population.

2. PARTICIPANTS AND METHODS

A total of 137 unrelated participants (62 women; 75 men) were enrolled in this cross-sectional study. The rs2576178 polymorphism was genotype in 100 patients on hemodialysis (48 normotensive and 52 hypertensive) and 37 controls (apparently healthy, normotensive individuals). Patients on hemodialysis were recruited from Clinic for Hemodialysis, Clinical Center, Sarajevo, but control participants were healthy volunteers selected mostly from medical staff. The study was approved by Ethic Committee Faculty of Medicine University of Sarajevo and participants gave informed consents. The patients and controls were originated from Bosnia and Herzegovina.

Hypertension was defined as having systolic blood pressure ≥140, diastolic blood pressure ≥90 according to diagnostic criteria (7) or being taking regular antihypertensive therapy.

To obtain genomic DNA for genetic testing we collected buccal cells with two swabs. Participant’s mouth was vigorously rubbed on the both sides of the cheek at least six times and swabs were placed inside of envelope. Used cotton swabs and the envelope were sterile. Upon receipt, the buccal swabs were placed and kept on room temperature to dry prior to DNA extraction. Genomic DNA was extracted from buccal swabs using a standard salting out procedure (Miller) (8).

Genotype Determination

Detection of the genetic polymorphism rs 2576178 was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and the MspI restriction enzyme. For determination of polymorphisms we used a pair of primers designed for the project: sense: 5’AGTGCCGTACCAGTAGTTAG’ and antisense: 5’GTGGGCTATTGTGGAGAA’ (Table 1).

The PCR reactions were performed in final volume of 30 µl using 50 ng of genomic DNA, 0.25 M of sense and antisense primer each, 1.5 mM of MgCl₂, and 200 µM of dNTP 2 U of Taq polymerase in 10X M PCR buffer, containing 100 mM Tris-HCl (pH 8.3) and 50 mM KCl (Applied Biosystems, Life technologies, Thermo Fisher Scientific Inc. 2015). Amplification of DNA was carried out in a Tpersonal Thermocycler (Biometra, Germany).

PCR conditions were as follow: an initial denaturation at 95°C for 1 min, 30 cycles of denaturation at 94°C for 45 s, annealing at 55°C for 30 s, and extension at 72°C for 45 s. The PCR was followed by a final step of elongation at 72°C for 7 minutes. The final step of DNA chain elongation lasted 7 minutes at 72°C.

Obtained PCR product by this procedure was 200 bp. Amplified products were digested with restriction enzyme MspI (TAKARA BIO INK, Japan) at temperature of 37°C for 2 hours. After digestion, wild-type AA genotype stays uncut, but fragments with mutant allele were cleaved and showed two bands of 160 and 40 bp (Figure 1). The genotypes were determined after electrophoresis separation on 2.5% agarose gels, staining with ethidium bromide and visualization by using UVItec Gel Documentation System (UVITEC Cambridge, UK).

Statistical analysis

Hardy–Weinberg equilibrium for alleles was tested using the chi-square test.

Genotype distribution and allele frequencies were assessed by a chi-square test of independence with 2 x 2 contingency tables and z-statistics. Statistical significance was defined as P<0.05. Statistical calculation was performed with SPSS for Windows (version 19.0. SPSS Chicago, IL).

3. RESULTS

The genotypes of renalse gene polymorphism rs2576178 (5’ flanking region) was determined in 137 participants (100 patients on hemodialysis and 37 controls). The mean age of hypertensive and normotensive patients were 53 (ranging from 23 to 65) and 62 (ranging from 18 to 79) years; respectively. Mean age of control group participants was 42 years (ranging from 16 to 61 years).

PCR products digested with MspI, were separated and visualized by electrophoresis in 2,5% agarose gel, stained with ethidium bromide. Lane 1: uncut PCR product; lanes 3 and 4: AA (homozygous wild-type genotype); lanes 2 and 6: AG heterozygous; lane 5: GG (homozygous mutated); lines M: 50 bp DNA ladder.
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For the rs2576178 polymorphism, the genotype distribution in all participants of study, was in Hardy-Weinberg Equilibrium (p=0.454). Also, distribution of studied genotypes in groups of normotensive patients on hemodialysis, hypertensive patients on hemodialysis treatment and control were in Hardy-Weinberg Equilibrium (p=0.712; P=0.457; p=0.855, respectively).

Genotyping results for the rs2576178 polymorphism are summarized in Table 2 and 3.

| Genotypes rs2576178 | Groups          | N-HD n(%) | H-HD n(%) |
|---------------------|-----------------|-----------|-----------|
| AA                  |                 | 14(29)    | 16(32)    |
| AG                  |                 | 25(52)    | 28(53)    |
| GG                  |                 | 9(19)     | 8(15)     |
| Total (n)           |                 | 48        | 52        |

| Genotypes | alleles | N-HD n(%) | H-HD n(%) |
|-----------|---------|-----------|-----------|
| A         |         | 55.0      | 58.0      |
| G         |         | 45.0      | 42.0      |

Table 2. The distribution of genotypes and alleles frequencies of rs2576178 renalase polymorphism in normotensive and hypertensive hemodialysis patients

AA- participants with homozygous wild-type genotype; AG- participants with heterozygous genotype; GG- participants with homozygous; A-wild-type allele; G-mutated allele; HD- hemodialysis; CG-control group; n-number of participants; (%)-percentage of genotype or allele in group of participants

Pearson chi-square test; genotype frequencies difference between N-HD and H-HD was at the level p=0.904 and allele frequencies difference was at the level p=0.671.

4. DISCUSSION

Hemodialysis patients are at higher risk of developing the high blood pressure. There are many factors contributing hypertension in this population: hypervolemia, increased sympathetic activity, erythropoietin and among them recently studied genetic factors. In the last decade, renalse and its role in blood pressure regulation have been studied extensively.

In this study we analyzed genotype and allele frequencies of renalase gene rs2576178 polymorphism in patients on hemodialysis and control group of healthy individuals in order to find out the possible association between renalase gene rs2576178 polymorphism and blood pressure in HD patients.

Browse the published papers for results of investigating the link between rs2576178 polymorphism in renalase gene and hypertension, showed the opposite findings. In Zhao et al (9) study it has been reported the association of renalase gene rs2576178 polymorphism and hypertension development in ESRD. Similarly, Fava et al (12) found no association between rs2576178 renalase gene polymorphism and hypertension in a Swedish cohort study. Case-control study of Ahiawat at al (13) revealed no significant difference among groups for genotype distribution and allele frequencies at SNP rs2576178 suggesting no association of renalse gene
rs2576178 polymorphism and hypertension, and chronic kidney diseases.

Different results have been published by Stec et al (14) who had been studied two renalse gene polymorphisms in ESRD patients affected by hypertension. They found higher risk of hypertension in ESRD patients who were carriers of G allele in both rs2576178 and rs10887800 renalse gene polymorphism. It has to be noted that healthy control was not examined in the Stec et al (14) study.

Limitations of this study were small sample size and subjects enrolled from one hemodialysis center. Genetic studies require large population.

5. CONCLUSION

Results couldn’t confirm association of renalse rs2576178 gene polymorphism and hypertension in patients on hemodialysis. Also, we observed no association of this renalse gene polymorphisms and renal function in population of Bosnia and Herzegovina.

Probably other genetic factors than renalse rs2576178 gene polymorphism play role in the hypertension in patients with ESRD on replacement therapy by hemodialysis, nevertheless this hypothesis requires further investigations.

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Investigation of Cardiac Complications and their Incidence in Patients with Ankylosing Spondylitis

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ABSTRACT

Introduction: Ankylosing Spondylitis (AS) is a chronic inflammatory disease with unknown etiology which involves the sacroiliac and axial joints, but can also cause peripheral conflicts. It also comprises non-joint symptoms such as acute anterior uveitis, cardiac conduction defects, upper lobe pulmonary fibrosis, neurological involvement and renal amyloidosis. Material and Methods: This study was a cross-sectional descriptive and analytical survey. In this study, 50 patients with AS were examined according to the New York Criteria in Army 501 Hospital in Tehran. Physical examinations, laboratory testing and HLA-B27, as well as X-ray of the spine and sacroiliac joint were taken from all subjects and involvement grading was identified. The control group consisted of 40 healthy people with no evidence of disease. The people resembled the study group in terms of age, sex, smoking, presence of high blood pressure, history of ischemic heart disease and also diabetes. Results: The mean age of patients in control and study group was 33.97 and 33.65 years, respectively. 37 (92.5%) patients in the control group and 46 in study group (92%) were male. The mean duration of cardiac involvement in patients was 8.6 years with SD=6.26. In AS group, 48 (96%) patients suffered from back pain, 43 from enteritis, 100% from Ankylosing Spondylitis, one from unilateral involvement, 22(44%) from peripheral arthritis and 27 (54%) from HLA–B27. Conclusion: In total, Average heart involvement in the control group and AS group was 13.25 with SD=7.64 and 16.2 with SD=8.54, respectively, indicating no significant difference. In sum, based on the results obtained in this study, some types of heart involvements, such as mitral valve regurgitation and Mitral Valve Prolapse in AS patients are more prevalent than in the normal population.

Key words: Cardiac involvement, Mitral valve, Ankylosing Spondylitis, Arthritis.

1. INTRODUCTION

Ankylosing Spondylitis is an inflammatory disease with unknown etiology causing chronic inflammation of the joints and surrounding structures of the spine. The inflammation may lead to focal bone erosions and new bone formation. In this condition, there is a possibility of peripheral joint involvement and also, the development of inflammatory lesions in Non-articular organs such as eyes, heart, lungs, kidneys and digestive system (1, 2).

The disease appears at young ages and predisposing family factors affect it and also there is a strong association with MHC genetic polymorphism (HLA-B27). The New York Criteria is used to diagnose Ankylosing Spondylitis in patients, so the same criteria has been used to classify Ankylosing Spondylitis in patients (3, 4). There is a significant correlation between the AS and histocompatibility antigens. Ankylosing Spondylitis disease affects men more than women (3:1) and the age of onset is typically from early...
adolescence to age 35 and peak at age 28 (5, 6). Family history can be seen in 20-15% of cases and the risk for HLA-B27 positive is almost 20% (7). No specific reason has been found so far and no environmental factor such as bacterial pathogens has been defined for it. However, Klebsiella pneumonia has 6 amino acids similar to HLA-B27, and it seems that molecular mimicry is effective in its role in causing the disease (8-13). It should be noted that pathogenesis is not fully understood for AS, but it is almost certainly immune mediated (14-16).

Clinical symptoms of the disease are chronic back pain and stiffness which are typical early signs and start gradually. The pain in Ankylosing Spondylitis has inflammatory nature, so that appears in the morning and resting time and improves followed by activity (2, 14). Enteritis, especially plantar tendon problem, may exist which will lead to heel pain. The first unusual symptom in clinical examinations in Ankylosing Spondylitis is usually tenderness in sacroiliac joint or pain in the same area with hip hyperextension. Findings from long-term observations showed that in the long term, cases such as flattening the normal lumbar lordosis curve and limiting the movements in all lumbar spine planes are visible. When the disease progresses towards thoracic spine, chest expansion is limited (under 2.5 cm), resulting from cost vertebral joint fusion, and considering as a specific sign for AS, especially in young people (2, 14).

In addition to detailed clinical symptoms mentioned, some symptoms can be also seen in other organs and out of joint, such organs as eyes, lungs, kidneys, digestive system, nervous system and heart (2, 14, 17). Aortic insufficiency and variable degrees of atroventricular block or branch block can be seen in approximately 5% of patients with a long period of illness. Less commonly, mitral insufficiency can be associated with aortic disease and almost all of the patients are HLA-B27 positive (18, 19). Valve involvement is histopathologically due to infiltration and accumulation of plasma cells and lymphocytes around the Vasa Vasorum, causing their lumen to be narrowed and the changes dilate and thicken the aortic root wall and shorten and thicken the aortic valve leaflets. Extension of inflammatory process and secondary fibrosis towards cardiac conduction system, namely, AV node and conduction branch proximal part, is the reason for conduction block (20-22).

In this study, the overall objectives are to investigate the frequency and type of cardiac involvement in patients referred to Army 501 Hospital, Tehran, and more specifically, frequency of cardiac involvement and complications in two groups of AS patients and healthy individuals, and finally comparing them and also examining the frequency of HLA-B27 and joint involvement in these patients.

2. MATERIALS AND METHODS

This study was a cross-sectional, descriptive-analytical survey, in which 50 patients with Ankylosing Spondylitis referred to Army 501 Hospital, Tehran, in 2001 - 2008 were compared with 40 healthy subjects as controls. This was a case-control study in which people with AS had been confirmed according to the New York Criteria. All patients, in addition to clinical examinations, underwent X-ray of the spine and sacroiliac joint. Also, all patients were referred to cardiologist to perform physical examination, electrocardiography and echocardiography during their visits. Control group was selected among healthy people having no signs of disease. The people were matched with the study group according to age, sex, smoking, high blood pressure, history of ischemic heart disease and also diabetes mellitus and finally referred to a cardiologist for electrocardiography and echocardiography. After collecting the information obtained and recording them, variables were analyzed using the software SPSS (Ver 15).

3. RESULTS

From total amount of patients in our sample, 50 AS patients were included in this study, 46 (92%) males and the remaining females. The control group consisted of 37 (92.5%) males and 3 (7.5%) females. The mean duration of disease in AS patients was 8.6 years, with 1 year minimum to 26 years maximum disease duration. The mean age of patients was reported 33.65 (SD=10.23) with age range of 20 to 63 years and age density between 20 to 40 years. In the control group, the mean age was 34 years (SD: 10.73) with minimum and maximum age of 20 and 63 years, respectively. Investigation of the frequency of HLA-B27 in patient group showed that 27 patients were HLA-B27 positive and 23 patients negative. Furthermore, the frequency of peripheral arthritis in the group indicated that 22 (44%) patients had peripheral arthritis and 28 (56%) were free of this complication. All AS patients had sacroiliac joint involvement, 49 (98%) bilateral and only 1 (2%) unilateral involvement. Based on back pain distribution, 48 (96%) patients had back pain and 2 (4%) were free of it.

Table 1: The frequency of different heart failure in AS patient and comparison with control group
The frequency of different types of heart failure were studied, including aortic stenosis, aortic regurgitation, mitral stenosis, mitral regurgitation, mitral valve prolapse, tricuspid valve stenosis, tricuspid valve regurgitation, pulmonary valve stenosis and pulmonary valve regurgitation, that the results can be seen in Table 1.

ECG changes in both groups of AS patients and controls were studied. In patient group, 46 (98%) subjects had no changes and only 1 (2%) subject showed ECG changes in favor of RBBB who was HLA-B27 negative. In control group, 36 (90%) subjects unchanged and 4 (10%) had ECG change. The changes were RBBB in 1, PVC in 2 and T-invert in 1 (Figure 1). Finally, since independent qualitative variable had two status (healthy group and AS patient group) and dependent variable was quantitative, T-test was used. Consequently, the average of this variable in AS patients and control group was 16.2±8.56 and 13.25±7.64. Because the significance level is \( P = 0.09 (>0.05) \), the main hypothesis is rejected, suggesting that being AS patient or healthy has no influence on cardiac involvement.

4. DISCUSSION

Ankylosing Spondylitis (AS) is a chronic inflammatory disease characterized by inflammation of the spinal cord and peripheral skeleton that can lead to local bone erosions or osteoporosis at the primary site. AS pathogenesis has not been fully recognized, but it is almost certainly immunity mediated. No laboratory test is diagnostic for AS. ESR and CRP are often high in most patients, but not always (23). Mild anemia may also be present. In severe cases, alkaline phosphatase level may also be high. On the other hand, in addition to peripheral joints and spine, other organs are also involved such as digestive system, lungs, eyes, kidneys, nervous system and heart. According to a recent study of AS patients, their cardiac involvement is divided into constructive involvement of heart itself including valves and vessels and cardiac conduction system. Valve involvements are as aortic or mitral insufficiency and conduction disorders as atrioventricular blocks or branch block that can be seen in approximately 5% of patients with long-term illness. Valve involvement is histopathologically due to infiltration of inflammatory cells, especially plasma cells and lymphocytes. Also, conduction disorders are led by inflammatory process and secondary fibrosis in conduc-

5. CONCLUSIONS

In conclusion, in total, according to data obtained in this study, it is suggested that the frequency of cardiac involvement in patients with AS doesn’t differ with general population. But some of them, such as mitral valve regurgitation and mitral valve prolapse, had higher prevalence in AS patients than normal population in our study. It should be noted that minimal involvement of aortic valve observed in this study can be caused by a short period of illness, so that in other studies, the mean duration was 17-16 years, but in our study it was 8.6±6.26 years.

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- **Conflict of interest:** none declared.
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The Effects of Total Motile Sperm Count on Spontaneous Pregnancy Rate and Pregnancy After IUI Treatment in Couples with Male Factor and Unexplained Infertility

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ABSTRACT

Introduction: Male infertility factor is defined if the total number of motile spermatozoa (TMSC) < 20 × 10⁶/ejaculated, and unexplained infertility if spermiogram is normal with normal female factor. The aim of this study was to determine the predictive value of TMSC for spontaneous pregnancy (ST) and pregnancy after treatment with intrauterine insemination (IUI) in couples with male factor and unexplained infertility. What is known already: According to the WHO qualification system abnormal spermiogram can be diagnosed as oligozoospermia (O), asthenozoospermia (A), teratozoospermia (T) or combination (O+A+T) and azoospermia (A). Although this classification indicates the accuracy of findings its relevance for prognosis in infertile couple and the choice of treatment is questionable.

Materials and Methods: The study included 98 couples with male infertility factor (bad spermiogram) and couples with normospermia and normal female factor (unexplained infertility). Testing group is randomized at: group (A) with TMSC > 3,10⁶ / ejaculate and a spontaneous pregnancy, group (B) with TMSC < 3 × 10⁶ / ejaculate and pregnancy after IUI, plus couples who have not achieved SP with TMSC > 3 x 10⁶ / ejaculate and couples who have not achieved pregnancy. Main results: From a total of 98 pairs of men’s and unexplained infertility, 42 of them (42.8%) achieved spontaneous pregnancy, while 56 (57.2%) pairs did not achieve spontaneous pregnancy. TMSC was significantly higher (42.4 ± 28.4 vs. 26.2 ± 24, p <0.05) in the group A compared to group B. Couples with TMSC 1-5 × 10⁶ ejaculate had significantly lower (9.8% vs. 22.2%, p <0.0001) rate of spontaneous pregnancy in comparison to couples after IUI treatment. Couples with unexplained infertility had significantly higher (56.8% vs. 29.9%, p <0.01) spontaneous pregnancy rate compared to couples after IUI treatment. Couples with unexplained infertility had significantly higher (56.8% vs. 29.9%, p <0.01) spontaneous pregnancy rate compared to couples after IUI treatment. Infertile couples had significant pregnancy rate with TMSC 5-10 x 10⁶ / ejaculate (OR = 1.45, 95% CI: 1.26-1.78, <0.01); with TMSC 10-20 x10⁶ / ejaculate (OR = 1.36, 95% CI: 1:12 to 1:46, <0.0001) with TMSC> 20 x10⁶ / ejaculate (RR = 1.7, 95% CI: 1.56-1.82, <0.001) after treatment with IUI compared to spontaneous pregnancy. Conclusion / Interpretation: Based on these results we can conclude that couples with the TMSC> 5 x 10⁶ / ejaculate are indicated for treatment with IUI. TMSC can be used as the method of choice for diagnosis and treatment of male infertility.

Key words: Total motile sperm count. Male infertility. Unexplained infertility. Intrauterine insemination. Spontaneous ongoing pregnancy rate.

1. INTRODUCTION

The World Health Organization (WHO) designated the infertility as a disease, and its treatment as one of the fundamental human rights. Infertility is defined as the inability
to conceive after 1 year of unprotected relations of couples in the reproductive age (1). Male infertility factor is the most common cause of not having children (2, 3). The diagnosis is based on the results of the analysis of semen. The WHO revised and defined cut-off value for differentiating normal and abnormal semen (4). Van der Steeg and his associates in 2011, tried to check the WHO criteria parameter analysis of seed in 1999 (5) for a spontaneous pregnancy, and it was concluded that the predictive value of the WHO classifications seed analysis was deficient. In other words, although the WHO proposed classification accuracy of seed analysis, relevance for prognosis and treatment choices are bad (6). The parameter for assessing the quality of sperm is the total motile sperm count (TMSC). The total number of mobile sperm has a better predictive value for spontaneous pregnancy (ST) then of the WHO classification system. Sperm morphology parameter is not used in this calculation (7). Van der Weert et al. (2004) showed in a meta-analysis of 16 studies that post wash TMSC between 0.8 × 10^6 and 5 × 10^6 has a prognostic value in couples who underwent IUI (8). Badawy et al. (2009) showed that the IUI is less successful when TMSC post wash is low with poor morphology (9). So, there is a need for new parameter values in seed analysis for clinical use in sorting out male infertility factors and selecting the treatment. The majority of the published studies show that IUI is more effective than planned sexual intercourse regardless of whether they are couples with unexplained infertility or male infertility. Intrauterine insemination shows a significant increase in pregnancy rates compared to the planned time sexual intercourse in natural cycles, regardless of the type of infertility (OR = 2.43) (10). The aim of this study was to determine the predictive value TMSC for spontaneous pregnancy (ST) and pregnancy after treatment with IUI in couples with male factor and unexplained infertility.

2. MATERIALS AND METHODS

Study design

Prospective study included examination of 223 infertile couples for marriage infertility with duration longer than 1 year. The study was conducted from January 2013 throughout December 2015 in the Department of Human Reproduction “Dr Hajder” Tuzla. Treatment of infertility was conducted in accordance with the guidelines for infertility (NICE) and consists of the examinations of both partners. All married couples underwent the following examinations: spermogram, analysis of sex hormones in the early follicular phase of the cycle, ultrasonic cycle monitoring (folliculometry), mid-luteinizing progesterone measuring, microbiological and immunological treatment of the infection, hystero sonosalpingography, diagnostic laparoscopy in indicated cases.

Study population

Total cohort consisted of 223 infertile couples. Of these, 115 pairs were excluded from the study due to female factor infertility, such as disorders of ovulation, tubal disease, endometriosis, cervical factor or sexual dysfunction. The study also excluded couples with a combination of female and male factors, couples in which the male partner had azoospermia and couples with TMSC <1 × 10^6 / ejaculate. The study included 98 couples with male infertility factor (bad spermogram) and couples with normospermia and normal female factor (unexplained infertility). The study group was randomized to: group (A) with TMSC> 3 x10^6 / ejaculate and a spontaneous conception; group (B) with TMSC<3 x 10^6 / ejaculate and conception after IUI, plus couples who did not achieve SP with TMSC> 3 × 10^6 / ejaculate and couples who did not achieve pregnancy. Both groups were classified in five subgroups based on TMSC subgroup (1) with TMSC 1-5 × 10^6 / ejaculate; subgroup (2) with TMSCI 5-10 × 10^6 / ejaculate; subgroup (3) with TMSCI 10-20 × 10^6 / ejaculate; subgroup(4) with TMSCI> 20 × 10^6 / ejaculate and subgroup (5) with unexplained infertility.

Definition. Infertility is defined as the failure of conception, despite 12 months of unprotected intercourse (Ciglar). Normozoospermia is defined according to the WHO criteria, 2010. TMSC is calculated by multiplying the concentration of sperm / milliliter (SC) x volume (ml) x motility (A + B) divided by 100%. Sperm is normal if TMSC > 20 × 10^6 / ejaculate, lower values are considered abnormal spermogram and define male infertility factor. Infertile couples with normozoospermia and normal female factor were considered unexplained infertility. The normal menstrual cycle is between 25 and 35 days, the level of progesterone in the medium luteal phase is ≥ 27 mmol / l, the duration of the luteal phase > 11 days.

Treatments

Couples with TMSC> 3x10^6 / ejaculate, with the chance of conceiving 30% (mild cases) were given an advice on planned sexual relations with ultrasound monitoring of follicular growth. Six IUI cycles were offered to couples with TMSC from 1 to 3 x 10^6 / ejaculate with a forecast of conception <30% (moderate male infertility), and couples with TMSC> 3 x 10^6 / ejaculate with a conception prognosis 30%, that did not achieve SP after 6 months of planned sexual intercourse. After six unsuccessful IUI cycles couples were offered in vitro fertilization (IVF) and were excluded from the study. Couples with TMSC <1 × 10^6 / ejaculate were immediately offered in vitro fertilization by intracytoplasmic sperm injection (ICSI) and were excluded from the study.

Outcome measures

The primary objective was to determine the rate of spontaneous pregnancies without treatment and pregnancy rates after IUI treatment. Pregnancy is defined by ultrasound measurement of the fetal heart beat after the 12th week of pregnancy, and increased h-bCG after 1-2 days of the period, the values of which should be doubled when re-measurement after 48-72 hours.

Semen analysis

The men had their spermogram made on arrival at the Institute. In the case of abnormal spermogram, a second sample was analyzed 10 weeks later. Male partners gave a semen sample after 2-3 days of sexual abstinence. The seeds were stored in the Institute in sterile plastic container, or at home and delivered to the laboratory of the Institute within 1 hour. During the analysis volume was measured by a numbered syringes. The sperm was read