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BMP4 circulating levels and promoter (rs17563) polymorphism in risk prediction of idiopathic male infertility

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Infertility is defined as a failure to achieve a pregnancy despite a year of unprotected regular sexual intercourse. The frequency of male infertility is about 15% of couples. Frequency of male factor contributing to infertility ranges from 18.8% to 39% [1]. Genetic anomalies, chromosomal disorders, mitochondrial DNA mutations, deletions in the Y chromosome or endocrine disruptors of genetic origin have been described in infertile men [2]. Infertility may also be associated with a wide range of semen anomalies, such as sperm number, motility and morphology [3].

Many genes are important in male infertility, testis formation and spermatogenesis involves progressive interactions between multiple cell types. Bone morphogenetic proteins (BMPs) are members of the transforming growth factor β (TGFβ) superfamily. BMP signalling has been shown to play important role in testis formation and fertility [4]. BMP4 has been shown to be expressed in testis and plays important roles in various aspects of embryonic development, and the major ligands involved in kidney formation, germ cell specification and spermatogenesis [5]. Moreover, BMP4 promotes DNA synthesis and proliferation of Sertoli cells and regulates the synthesis of some proteins essential for spermatogenesis via second messenger pathways. In addition, a clear association between the abnormal expression of BMP4 and different subtypes of azoospermia patients has been reported [6], whilst BMP signalling disregulation could increase the occurrence of apoptosis of spermatogenic cells in the testes suggesting that BMPs play role in the progress and maintenance of spermatogenesis [7]. The present study tested the hypothesis of a link between BMP4 rs17563 gene polymorphism and serum circulating levels in idiopathic male infertility.

One hundred idiopathic infertile men (aged mean 36.2 standard deviation 11.5 years) and 126 fertile men (37.1 [13.6] years) (P = 0.695) were recruited. At least three seminal fluid analyses, carried out after 3–5 days of sexual abstinence were performed to determine their infertility status. Semen analysis was performed according to World Health Organization recommendations, 2010 [8]. Patients with a positive history of epididymo-orchitis, prostatitis, genital trauma, cryptorchidism, chromosomal abnormalities, testicular torsion and bilateral absence of the vas deferens, varicocele, hypogonadotropic hypogonadism, seminal infections, drug, alcohol user, and chronic diseases such as diabetes were excluded. All patients provided a medical history and underwent physical examinations. A control group was healthy men with normal semen parameters who had at least one child without using assisted reproductive technologies. Each subject provided 3-ml blood, drawn into EDTA-coated tubes for genomic DNA extraction. Serum samples were also collected, centrifuged at 9000 rpm for 6 min and the serum frozen immediately and stored at −70 °C. Informed consent was obtained from all participants. The study was approved by the university ethical committee and was in accordance with the 1964 Helsinki declaration.

Genotyping of BMP4 T152C (rs17563) was determined by PCR-RFLP method using *Hph1* restriction enzyme and the following primers: forward 5′- TTC ACCATTCATTGCCCAACC-3′ and reverse 5′-GAAGC CCCTTTCCCAATCAGG-3′ which have been designed by Oligo-primer analysis software (Version 7.54, Molecular Biology Insights, USA). BMP4 serum levels have been measured using Human BMP4 ELISA Kit (ab99982) (Abcam, Cambridge, UK) and antiserum against human BMP4 (n = 35 for each groups). The odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. The relationship was regarded as significant when they reached $p < 0.05$. Statistical Package for the Social Sciences (SPSS) version 20.0 (Chicago, IL, USA) was used for data analysis.

Genotyping of T152C of BMP4 was successfully performed in all subjects. The sizes of bands produced by

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electrophoresis were determined by comparison with standard DNA ladder. Genotype frequencies of the BMP4 (rs17563) single-nucleotide polymorphisms (SNPs) did not deviate from Hardy-Weinberg equilibrium (HWE) in controls (χ^2 = 1.088; p = 0.29). A significant difference was found between genotype and allele frequencies of the BMP4 polymorphism in the controls and the patients determined by the expected value of the χ^2-test (P = 0.005; P = 0.008, respectively). The BMP4 genotype frequencies amongst the infertile men were C/C = 27%, T/C = 18%, T/T = 55% and in controls were C/C = 6.3%, T/C = 31%, T/T = 55% (P = 0.005). In a co-dominant model (C/C vs. T/T genotype), the C/C genotype was linked with a higher risk of infertility when compared with that of the T/T genotype (OR = 4.84; 95%CI = 2.04–11.49; p = 0.003). The recessive genetic model revealed a significantly increased risk of idiopathic male infertility in C/C genotype carriers when compared with that of the T/T and T/C genotypes (OR = 5.45, 95%CI = 2.35–12.65, p = 0.001). The C allele was overrepresented in cases (36% versus 22%, in controls) leading to two-fold increase risk of idiopathic male infertility (OR = 2.01; 95%CI = 1.32–3.05; p = 0.001). The mean [SD] levels of serum BMP4 in the infertile men (12.0 [3.2] pg/ml) was significantly lower than in controls (18.3 [4.8] pg/ml) (P = 0.007) (Figure 1).

Our data support the hypothesis of a link between the BMP4 (rs17563) polymorphism and its serum levels that is significantly associated with an increased risk of idiopathic male infertility. The minor allele frequency is reduced in control group compared to cases and there was two-fold higher risk in cases compared to controls. We have also showed that the BMP4 serum concentration was significantly decreased in the men with idiopathic male infertility as compared to fertile men. The rs17563 polymorphic site is one of the most functional SNPs of BMP4. This SNP showed the change from T to C at 538 nucleotide position (538T/C) [9]. The rs17563 polymorphism results in an amino acid change from valine to alanine at residue 152 (T152C) that affects the *BMP4* gene expression. The T-allele was predicted to change mRNA structure and the BMP4 mRNA levels were significantly higher in T-allele carriers compared with C-allele carriers [10]. It has been shown that single nucleotide polymorphism of BMP4 rs17563 is significantly associated with the incidence of left ventricular hypertrophy in hypertensive patients [11]. The association of many gene polymorphisms was shown to be associated with male infertility. The association between catalase and GDNF (rs2075680) SNP with male infertility has been reported [12,13]. The impact of numerous BMPs on the survival and proliferation of murine germ cells during embryonic development and the regulation of postnatal germ cell and somatic cell populations has been identified from *in vitro* and *in vivo* studies [5]. Serum BMP-4 concentrations are related with adiposity and Type 2 Diabetes [14]. The significant association between BMP4 rs17563 C mutation and the risk of nosyndromic cleft lip with and without palate (NSCL/P) has been reported [15].

The results of this research should be interpreted in view of limitations. Firstly, the size of sample is relatively small; the result should be interpreted with caution. Secondly, data may not be extrapolable to other populations. Thirdly, since only one SNP of BMP4 has been examined in this study, we cannot exclude the possibility that other genetic variants could play a role in idiopathic male infertility susceptibility. Finally, many factors

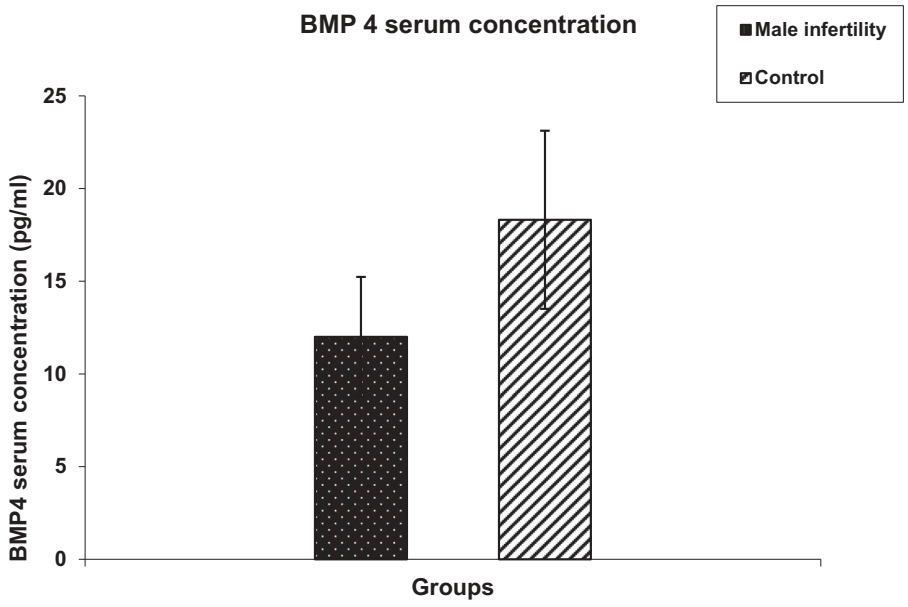

Figure 1. Mean (SD) BMP4 serum level in controls and men with idiopathic infertility. Significant decrease in serum BMP4 level was seen in the idiopathic male infertility samples compared with the control group (n = 35/group).

act individually and together to influence risk of male infertility. Therefore, more factors should be included in our future research.

This work represents an advance in biomedical science because it shows a link between of the BMP4 T152C (rs17563) polymorphism and idiopathic male infertility, and so warrant the inclusion of the tests as part of a routine clinical service.

Disclosure statement

No potential conflict of interest was reported by the authors.

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