Association of trinucleotide repeat polymorphisms CAG and GGC in exon 1 of the androgen receptor gene with male infertility: a cross-sectional study

Background: Infertility is a global problem that brings with serious sexual and social consequences that strain the health sector and society. The expansion of CAG & GGC repeats in androgen receptor (AR) gene Ensembl number; ENSG00000169083 may leads to reduced fertility. Our objective was to determine association of CAG and GGC repeats with altered sperm parameters in male infertile subjects.

Materials and Methods: It was a cross sectional study conducted at Aga Khan University, Karachi Pakistan. A total of 376 males were recruited, out of which group A (N=208) and group B (N=168) comprised of subjects with normal and altered sperm parameters respectively from 18 to 60 years. Numbers of CAG and GGC repeats were determined by using PCR amplification and sequence analysis using Molecular Evolutionary Genetic Analysis (MEGA) software version 6.0. Statistical analysis was done using SPSS version 20 and p-value of < 0.05 were considered significant.

Results: The mean androgen receptor gene CAG repeats were significantly longer in males with altered sperm parameters as compared to male subjects with normal sperm parameters (p< 0.001). There was no significant difference found for GGC repeats for subjects with altered sperm parameters.

Conclusions: Longer CAG length corresponded to greater severity of spermatogenic defect and may lead to subfertility recommendations.

Key words: Androgen receptor, trinucleotide repeats, male infertility
1. Introduction

The WHO defined infertility as “a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse” [1]. Around the world, research on infertility has focused on female infertility, but recent shift in healthcare dynamics have necessitated a fresh look into male infertility and the corresponding factors that influence its extent, severity and prevalence. Male infertility in 50% of the cases is idiopathic owing to the fact that human spermatogenesis is controlled by several hundreds of genes [2].

The environmental causes of male infertility include prolonged exposure to heat [3], noise pollution [4], ionizing radiation [5], air pollution [6], mumps after puberty [7], alcoholism [8] and psychological stress [9].

The genetic causes underlying male infertility include: Y chromosome microdeletions [10], sex chromosome aneuploidies such as Klinefelter’s Syndrome [11] and gene polymorphisms such as those in LOC203413 or CAG repeats in androgen receptor gene [12].

Androgen is indispensable for the male sex differentiation and spermatogenesis [13], testosterone and dihydrotestosterone are physiological androgens that play an important role in development of male external and internal genitalia [14]. Androgens act through a steroid receptor known as androgen receptor which is encoded by an androgen receptor gene, a ligand dependent nuclear transcription factor [15]. The androgen receptor (AR) gene, Ensembl number; ENSG00000169083 is located on chromosome Xq11-12 has 8 exons and 7 introns, out of which exon 1 has CAG and GGC repeats and is associated with transcription [14,16].
Polymorphic trinucleotide repeat segment, (CAG)n; which encodes a polyglutamine tract in which n usually ranges from 11 to 31. This CAG repeat tract has been the basis of unprecedented attention in current years because it has been found that expansion of CAG segment to more than 40 repeats leads to spinal bulbar muscular atrophy, a mortal neuromuscular disease. Trinucleotide repeat expansions are now associated in many diseases [14].

AR gene is susceptible to one of the largest number of mutations in a steroid receptor, documented at up till 300 mutations [17]. The AR gene essentially consists of three main functional domains: N-terminal transcriptional regulation domain, DNA-binding domain and ligand binding domain. Androgens bind to AR gene and exert their actions either through DNA binding dependent manner, regulating gene transcription, or non-DNA binding dependent manner, causing phosphorylation of secondary messenger system [18]. The AR gene is expressed on a multitude of tissues in the body such as prostate, bone, adipose tissue, muscle, etc and plays an important role in immune, hematopoietic, respiratory, cardiovascular and neural systems [19,20].

CAG trinucleotide repeat length normally is between 16 and 29 [21]. The variation in length of CAG repeats is between 6 and 39, with African Americans having an average length of 19 to 20 repeats, Caucasians having 21 to 22, Asians having 22 to 23 and Hispanics having 23 repeats [22]. CAG, located within the N-terminal transactivation domain, encodes polyglutamine and the number of glutamine encoded is significant for the function and structure of the AR gene [23] since the number of CAG triplet repeats determines the variability of the size of the androgen receptor [24].
AR resistance is reported to account for 40% of male infertility cases [25]. Increased CAG length in AR gene has been correlated to male infertility owing to decreased transcription of AR gene and diminished spermatogenesis [26]. Therefore an inverse relationship exists between longer CAG repeat length and the transcriptional activity of AR gene [27]. Most of the infertile males with increased CAG length presented with azoospermia [13]. Even though studies show that shorter CAG repeat lengths such as in African Americans predisposes them to developing prostate cancer, it has been reported that increased CAG repeat length have been attributed to male infertility cases among Asians and Caucasians [22,28,29]. Increased CAG length of > 26 nucleotides is linked to a seven fold increased risk of infertility [30]. Increased CAG repeat length is also associated with depression and reduced potency in males and CAG repeat length greater than 40 is known to be linked to Kennedy’s disease, a fatal neuromuscular disease [31,32]. However, the exact molecular mechanism behind CAG repeat polymorphism along with the association between length of CAG repeats and the severity of disease requires further study [33].

The transactivation domain of AR also contains a polyglycine tract, encoded by GGT/GGC six glycine tract proceeded by a polymorphic GGC repeat of length 10 to 35 nucleotides. In spite of limited studies showing the association between GGC repeat polymorphism and male fertility, there have been certain studies [34] showing an association between GGC and AR gene protein levels, suggesting that GGC repeat length of 13 produced 2.7 times more AR protein than did GGC repeat length of 17. Shorter GGC repeat length has also been linked to prostate cancer [35]. Research shows that both long CAG and GGC repeats have a negative impact on transcriptional activity of AR gene, while also having an association with the prostate and endometrial cancers [36]. Hence, we are interested in
finding the association between the lengths of the CAG and GGC repeats in the AR gene and altered sperm parameters in our population of male subjects with infertility problems.

2. Materials & Methods

This cross-sectional study was conducted from July 2017 to July 2018 at Department of Biological and Biomedical Science, Aga Khan University, in collaboration with ‘Sindh Institute of Reproductive Medicine” after acquiring Ethical Approval of the institutional ethical review board (2019-1226-3970). Sample size was calculated on Open Epi software version 3.01. A sample size of 398 was estimated to find the CAG and GGC repeat polymorphism in both groups adjusting for 10% non-response rate. We assumed a level of significance of 5%, Odds ratio of 2 and power for detecting the true effect of 80%. However, final recruitment was of 376 males on account of a number of refusals to provide semen samples.

'Patient and Public Involvement'. All male subjects were recruited from “Sindh Institute of Reproductive Medicine” based on non-conception after regular, unprotected intercourse for a period of at least 1 year. Possible genetic causes of male infertility were excluded by Y chromosome microdeletions and Karyotype analysis. The infertile subjects were divided into two groups (A and B) on the basis of normal or altered semen parameters (respectively). All subjects were collected via convenience sampling after obtaining written and informed consent.

At the time of recruitment, a data collection form was filled to record clinical data such as age, height, weight, calculation of Body Mass Index (BMI), estimation of body fat percentage, blood pressure, smoking habits, hormonal treatment and clinical history. Group A (normal sperm parameters) comprised of male subjects, with sperm count more than 39 ×
10^6, sperm motility of more than 50%, and normal morphology ≥ 4% with age ranging from 18 to 60 years from all ethnic backgrounds [37] were included. Male Subjects who were smokers, had diabetes, hypertension, or any serious health condition like myocardial infarction or are under hormonal treatment were excluded from study. Male subjects who had sperm count less than 39 × 10^6, sperm motility less than 50% and normal morphology ≤ 4% [37], between the ages of 18-60 year from all ethnic backgrounds comprised Group B. The exclusion criteria was kept as the same for infertile subjects.

Blood sample was collected for DNA extraction from ante-cubital vein in two tubes, serum gel tube (3 ml) and EDTA tube (2 ml). Serum was centrifuged at 3500 rpm for 5 minutes, separated into two aliquots and stored at -70 °C. DNA was extracted from 2 ml blood in EDTA tube using standard protocol of Promega Genomic DNA Extraction Kit Cat# A1125. Steps included: taking 6 ml of cell lysis solution with 2 ml blood (centrifuging at 3500 RPM for 10 mins.), adding 2 ml nuclei lysis solution and 660 µl protein precipitation solution (centrifuge at 3500 RPM for 10 mins), transfer supernatant to tube of 2 ml cold isopropanol, aspirate and add 200 µl rehydration solution, incubating for 24 hours. at 4 °C, vortex before aliquoting extracted DNA and keeping it at -80 °C for further analysis. The extracted DNA was quantified by measuring the ultraviolet absorbance and determining the absorbance ratio (A280/A260) for 2 µL samples, using a Nanodrop-ND1000 (Thermo Fisher Scientific, Waltham, MA). Extracted DNA was considered pure at an absorbance ratio of ~1.8.

Primers were designed using Primer 3 output primer designing tool for CAG (forward: 5’-TCCAGAATCTGTCCAGAGCGTGC-3’, reverse: 5’-
GCTGTGAAGGTTGCTGTTCCTC-3") and GGC (Forward: 5’-
ACAGCCGAAGGAAGGCCAGTTGTAT-3’, reverse: 5’-
CAGGTGCGGTGAAGTCGCTTTCCT-3’) repeat region of AR gene. Polymerase Chain
Reaction (PCR) for CAG repeats was performed using 2× PCR Hotstart Master Mix (Cat#
G906, ABM (Applied Biological Materials Inc, Canada) as per the manufacturer’s
instructions. PCR conditions were 1 cycle for 5 min at 95 °C for initial denaturation
followed by 30 cycles at 95 °C for 30 s, 60°C for 45 s, 72 °C for 45 s followed by a final
extension of 10 min at 72 °C. PCR was performed for GGC repeats using GoTaq hot start
master mix (Cat# M5122, Promega, USA) as per the manufacturer’s instruction. PCR
conditions were 1 cycle for 5 min at 95 °C for initial denaturation followed by 30 cycles at
95 °C for 30 s, 50°C for 45 s, 72 °C for 45 s followed by a final extension of 10 min at 72
°C. The amplified products were electrophoresed on 2% agarose gel. Purification of the
PCR products was done using PCR Clean Up for DNA Sequencing (Cat. No BT5100, Bio
Basic Inc, Canada) following the manufacturer protocol.
Sanger sequencing is classical method for sequencing and CAG polymorphism in AR gene
can be detected by PCR amplification and direct sequencing in published article [14]. This
method was utilized to sequence the AR gene in samples of Group A and B and PCR
products were sent to sequencing company Operon (Canada). Samples were sequenced to
obtain accuracy in finding the number of repeats in polymorphic trinucleotide repeat
segment of AR gene. The obtained sequences were directly compared to previously
published CAG and GGC repeat region of AR gene sequence using the MEGABLAST
search tool in the National Center for Biotechnology Information (NCBI) database.
Sequence files were imported into Chromas Lite, and then assembled using Molecular Evolutionary Genetic Analysis (MEGA) version 6.0.

Statistical analysis was performed with SPSS version 20 software by Mann-Whitney U test, and Spearman’s rank correlation tests (p-value of < 0.05 considered significant). Logistic regression analysis with 95% confidence intervals (CIs) was performed to report odds ratios (OR). Sequences were analyzed using Molecular Evolutionary Genetics Analysis (MEGA) software version 6.0.

3. Results

A total of 376 males presenting to infertility Centers were enrolled in the study. Subjects (168) in Group A had normal semen parameters, whereas 208 male subjects in Group B had abnormal semen parameters.

Table 1 presents the demographic characteristics of both groups. The mean age among Group B was significantly higher 37.4 ± 7.1 as compared to Group A subjects 35.5 ± 6.4 (p< 0.001). The mean BMI was significantly higher among altered sperm parameters (Group B) as compared to subjects with normal sperm parameters (Group A) 27.6 ± 2.6 vs 24.6 ± 3.2 (p< 0.001). The mean body fat % was also significantly higher in Group B as compared to Group A 34.5 ± 4.7 vs 32.9 ± 4.1 (p< 0.001).

Table 2 presents the semen characteristic of subjects in both groups. The mean total count and morphological normal sperms was higher among Group A as compared to Group B; 97.2 ± 30.2 vs 33.4.98 ± 22.6 (p< 0.001) and 8.2 ± 4.1 vs 3.5 ± 2.3 (p< 0.001) respectively. The motility was lower among Group B as compared to Group A subjects with median (IQR) 41 (25-46) and respectively 75 (70-78) (p<0.001). A higher proportion (41.3%) of Group B had Teratozoospermia followed by astheno Teratozoospermia (16%), azoospermia
(16%) and Severe Oligoasthenoteratozoospermia S.O.A.T (15.4%) and lastly Oligoasthenoteratozoospermia O.A.T (10.6%)

Table 3 showed the logistic regression analysis for group B which includes males with altered sperm parameters after adjusting with age and BMI. Males who have shorter CAG length (< 26) are considered as baseline to find out odds ratios for sperm count, motility and morphology for males having longer CAG(> or = 26). With every one unit increase in sperm count, the prevalence of infertility was decreased by 5% (p< 0.001), however, with every one unit increase in sperm motility and morphology, the prevalence of infertility was decreased by 18% and 4% respectively (p< 0.001). Considering ‘Altered Sperm Parameters’ as the factor contributing to subfertility (Table 4), the results of association between CAG length and male infertile subjects of Group B having longer (> or = 26) and shorter CAG length (< 26) is outlined. The sperm count with CAG length (< 26) was 36 million/mL which was significantly higher than the sperm count with longer CAG lengths (> or = 26) which was 30 million/ml (p= 0.001). Among those with shorter CAG lengths (< 26), the sperm motility was 35%, which was significantly higher than the sperm motility among those with longer CAG lengths (> or = 26) which was 21% (p= 0.002). Among those with shorter CAG lengths (< 26), the sperm morphology was 3% which was comparable with longer CAG lengths (> or = 26) which was 2%.

The mean values of CAG lengths in infertile men with altered sperm parameters (27) was significantly higher than the ones who had normal sperm parameters (24) shown in Figure1. There was however no significant difference observed between GGC repeat polymorphism in male subjects with Normal(A) and altered sperm parameters (B).
Figure 2A and B presents gel electrophoresis images of amplified PCR products for CAG repeats (band size = ~288bp) and for GGC (band size = ~184bp) in infertile and fertile males. Figure 2 C and D presents the sequencing chromatograms of CAG and GGC repeat polymorphism regions of AR gene.

However, GGC length, alone, on the other hand, did not show any significant association with male infertility as demonstrated by our results. The mean values of GGC length in Group B subjects (17) was comparable in Group A (16) subjects. Figure 2B shows that the most frequently reported GGC length among both A and B group subjects was 17, which showed no difference between the two groups.

4. Discussion

The increased prevalence of infertility with psychological and economic burden of developing countries has motivated researchers to look for relationship of infertility with genetic factors. The main observation in this study was that infertile males have significantly longer CAG length compared with fertile male subjects.

Increased fat in the scrotum leads to the production of gonadal heat which impairs sperm production since sperms need optimum temperature of 34-35 degrees. This accounts for the higher BMI among infertile men compared to fertile men in our study. Heat production causes oxidative stress to sperm, fragmentation of sperm DNA and diminished sperm motility [38].

Increased CAG tri-nucleotide repeat length has been associated with male infertility [39]. This is in contrast to few studies such as Nenonen et al 2011 that do not demonstrate a significant association between CAG repeat lengths and male infertility [40]. CAG tri-nucleotide length influences the functioning of the androgen receptor gene [41]. CAG tri-
nucleotides are located within the N-terminal trans-activation domain and encode polyglutamine and the number of glutamine determines the function and structure of the AR gene [23]. Increased CAG tri-nucleotide lengths are associated with diminished action of the androgen receptor whereas decreased lengths of the CAG tri-nucleotide are associated with enhanced action of the androgen receptor [42]. Shorter CAG lengths, on the other hand, are demonstrated by studies such as Gomez et al 2016 to be associated with early onset and sporadic prostate cancer. Longer CAG lengths lead to decreased transcriptional activity of the androgen receptor gene and subsequently impair spermatogenesis [24]. Thus, our results show a higher mean value of CAG length among infertile men compared to fertile men.

The total sperm count and sperm motility of the infertile men recruited in our study was reported to be lower than that of the fertile men recruited, which corroborates the negative effect of increased CAG length (>26) on sperm parameters. Furthermore there are studies such as Mengual et al 2003 show that men with greater than 26 CAG repeats had a risk of being azospermic [43]. Xiao et al 2016, on the other hand, shows the association between longer CAG lengths and oligospermia, not azoospermia or severe oligospermia. Delli Mutti et al 2014 demonstrates that increased CAG length causes decreased sperm motility, which was proven by our results [44]. Pan et al 2016 shows the inverse relationship between CAG lengths and male infertility among Asian, Caucasians and mixed races, in conjunction to demonstrating the increased risk of azoospermia with longer CAG lengths [21]. Even though the difference between the sperm morphology among infertile men and fertile men in this study was not statistically significant, there are studies such as Milatener et al 2004
that demonstrate a positive correlation between abnormal sperm morphology and increased CAG lengths [45].

However, GGC length, alone, on the other hand, did not show any significant association with male infertility as demonstrated by our results. Even though literature such as Gao et al 1996 shows that deletion of poly-glycine tract and reduction of androgen receptor transcriptional activity, there is little evidence to prove the impact of GGC length and male infertility [46,47]. Studies such as Ferlin et al 2005 show the combined effect of CAG and GGC in terms of causing male infertility. Furthermore CAG/GGC haplotypes have demonstrated an association with male infertility, as reported by Ferlin et al 2005 [13]. The aforementioned article also shows that CAG repeats and GGC repeats individually were not significantly different among those with azoospermia, mild or severe oligospermia however the haplotype CAG/GGC was more reported among those with low sperm count. Therefore, there is a need to explore the association between GGC lengths alone and their impact on male infertility. Moreover, the CAG repeats in AR gene is a polymorphism that may be associated (not causal effect) with male infertility, and its clinical relevance is still debated. Limitations of the study are that the occupational status of each subject was not determined and we did not correlate smoking status with polymorphism. All triplet repeat disorders show anticipation and a significant correlation between age at onset of the disease and length of the expanded repeat [48,49]. Current data is insufficient to conclude whether IVF patients who display AR CAG expansion may transfer infertility to their descendants. This CAG triplet repeat disorder (Group B) however is anticipated to be expressed in future generations in terms of alteration in sperm count, motility and morphology, hence contributing to male infertility. AR CAG polymorphism is not recommended in the routine
setting, yet the test may become imperative on the basis of clinical relevance, pharmacogenetic implications, theoretical possibility of transmission to next generation and tailoring of testosterone replacement therapy on the basis of length of CAG repeat in hypogonadal men.

Longer AR CAG repeat lengths cause decreased sperm motility, corresponded to greater severity of spermatogenic defect that can lead to disturbance in reproductive functions thus causing male infertility. However, GGC did not reflect any association with male infertility.

Acknowledgement

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References


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<table>
<thead>
<tr>
<th>Variables</th>
<th>Group A Normal Sperm Parameters (n=168)</th>
<th>Group B Altered Sperm Parameters (n=208)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) (Mean ± SD)</td>
<td>35.5 ± 6.4</td>
<td>37.4 ± 7.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (in kg/m²) (Mean ± SD)</td>
<td>24.6 ± 3.2</td>
<td>27.6 ± 2.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (in) 18.5 - 24.99 kg/m²</td>
<td>73(43.5%)</td>
<td>42 (20.2%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (in) 25 - 29.99 kg/m²</td>
<td>95(56.6%)</td>
<td>166(79.8%)</td>
<td></td>
</tr>
<tr>
<td>Body fat % (Mean ± SD)</td>
<td>32.9 ± 4.1</td>
<td>34.5 ± 4.7</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Body fat % &lt;25</td>
<td>4 (2.4%)</td>
<td>3 (1.4%)</td>
<td>0.32</td>
</tr>
<tr>
<td>Body fat % 25-29.99</td>
<td>36(21.4%)</td>
<td>29 (13.9%)</td>
<td></td>
</tr>
<tr>
<td>Body fat % &gt;29.99</td>
<td>128(76.2%)</td>
<td>176(84.6%)</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± standard deviation. Group A and B were compared using Mann Whitney test. *p-value < 0.05 were considered as significantly different.
Table 2: Semen characteristic of Study Groups

<table>
<thead>
<tr>
<th>Factors</th>
<th>Group A Normal Sperm Parameters (n=168)</th>
<th>Group B Altered Sperm Parameters (n=208)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total count (in mill/ml) Mean ± SD</td>
<td>97.2 ± 30.2</td>
<td>33.4 ± 22.6</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Motility Median (IQR)</td>
<td>75 (70-78)</td>
<td>41 (25-46)</td>
<td>&lt;0.001*#</td>
</tr>
<tr>
<td>Morphology Mean ± SD</td>
<td>8.2 ± 4.1</td>
<td>3.5 ± 2.3</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

### Morphological forms

| Normozoospermia                               | 135(100.0%)                            | 0(0%)                                   | <0.001*^    |
| Teratozoospermia                              | 0(0%)                                  | 86(41.3%)                               |
| Azoospermia                                  | 0(0%)                                  | 34(16.3%)                               |
| Severe                                       | 0(0%)                                  | 32(15.4%)                               |
| Oligoasthenoteratozoospermia (S.O.A.T)       | 0(0%)                                  | 34(16.3%)                               |
| Astheno Teratozoospermia                     | 0(0%)                                  | 22(10.6%)                               |
| Oligoasthenoteratozoospermia (OAT)           | 0(0%)                                  |                                         |

| Rapid linear Progression Median (IQR)         | 1.00(0-2)                              | 0.00 (0-1)                              | 0.015*#     |

*Significant at p value <0.05 (Independent t test for quantitative variables was used)

*^ Significant at p value <0.05 (Fishers exact test was used)

*# Significant at p value <0.05 (Mann Whitney test was used)
Table 3: Effect of sperm characteristics on chances of Subfertility

<table>
<thead>
<tr>
<th>Factors</th>
<th>Odds Ratio</th>
<th>95% Confidence Interval</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Sperm Count (mill/ml)</td>
<td>0.995</td>
<td>0.992-0.999</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sperm Motility (%)</td>
<td>0.982</td>
<td>0.977-0.997</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sperm Morphology (%)</td>
<td>0.960</td>
<td>0.952-1.024</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Significant at p-value <0.05 (Logistic regression analysis was performed to find Odds ratio and 95% confidence interval for risk of male subfertility).

Table 4: Association of Sperm parameters with CAG length in infertile males with Altered Sperm Parameters (n=208)

<table>
<thead>
<tr>
<th>Factors</th>
<th>CAG (&lt;26) n=96</th>
<th>CAG (≥26) n=112</th>
<th>rho value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Sperm Count (mill/ml)</td>
<td>36.4 ± 24.4</td>
<td>30.1 ± 21.4</td>
<td>-0.444</td>
<td>0.001*</td>
</tr>
<tr>
<td>Sperm Motility (%)</td>
<td>35.4 ± 21.04</td>
<td>21.3 ± 12.1</td>
<td>-0.326</td>
<td>0.002*</td>
</tr>
<tr>
<td>Sperm Morphology (%)</td>
<td>3 ± 1.6</td>
<td>2 ± 0.96</td>
<td>-0.068</td>
<td>0.64</td>
</tr>
</tbody>
</table>

*Significant at p-value <0.05 (Spearman’s rank correlation was used to find an association of sperm parameters with CAG repeat polymorphism.)
Figure 1: Comparison of mean values of CAG and GGC repeats in Normal & Altered Sperm Parameters.

* shows that there was significant difference p-value <0.05 between the Group A (normal sperm parameters) and Group B (altered sperm parameters) for CAG repeat polymorphisms. Using Mann-whitney test.
Figure 2: Gel electrophoresis and sequence chromatograms of CAG and GGC repeats in male subjects (Group B).

Figures 2 (A-D) Gel electrophoresis and sequencing chromatograms of CAG and GGC repeat polymorphism. Where M is the 100bp DNA marker. A & B: PCR amplification shown as bands on 2% agarose gel of CAG repeats (~288bp) and GGC repeats (~184bp) respectively of male samples of Group B numbered 1-12. C & D: Sequencing chromatograms showing the CAG and GGC repeats respectively.