ORIGINAL ARTICLE

ANTIOXIDANT QUERCETIN LEVELS IN SUB-FERTILE MEN AND THEIR ASSOCIATION WITH OXIDATIVE STRESS

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Background: Oxidative stress contributes significantly to male sub-fertility, underscoring the importance of antioxidants in improving semen quality. The primary objective of this study was to evaluate *in vivo* levels of antioxidant quercetin in sub-fertile men. **Methods:** This cross-sectional study was conducted over a period of 6 months at Imran Idrees Teaching Hospital Sialkot, Pakistan. The study population consisted of 44 males, divided into two groups: 11 healthy fertile controls and 33 sub-fertile men. The study group was further subdivided into those diagnosed with varicocele (n=11) and those without varicocele (n=22). Semen samples were collected following 3–4 days of abstinence in a sterile environment through masturbation. Semen analysis was performed according to WHO criteria, and sperm morphology was assessed using Kruger's strict criteria. Quercetin levels in semen were measured using High-Performance Liquid Chromatography (HPLC). Data analysis was conducted using SPSS, with One-way ANOVA employed for variable comparisons and Scheffe's test for correlation studies. **Results:** Healthy controls had higher sperm counts (80.09±5.40 million/mL) and motility (77.73±3.72%) than sub-fertile men with varicocele (14.00±4.02 million/mL, 30.18±7.13%) or without varicocele (23.55±3.17 million/mL, 34.77±6.51%) (p<0.001). Quercetin levels were significantly higher in controls (865.84±227 µg/mL) compared to study group (p<0.05). **Conclusion:** This study confirms the presence of quercetin in semen and demonstrates its positive correlation with sperm parameters related to fertility. Diet rich in the antioxidant quercetin may enhance male fertility, particularly in men seeking treatment for sub-fertility.

Keywords: Antioxidant, Oxidative Stress, Quercetin, Sub-fertile, Infertility

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INTRODUCTION

Infertility problem is a serious concern in our society. Approximately, 8–12% couples are affected out of which half can be due to either partner. Infertility has been defined as an incapability of a couple to conceive after having a regular unprotected intercourse for one year.[1]

Male sub-fertility is due to disruption in spermatogenesis progression normally. It is presently known that sub-fertile men have numerous structural and functional defects of spermatozoa. Thermal harm to the proteins and nuclear deoxyribonucleic acid (DNA) of spermatozoa is possibly the reason for change in spermatogenesis. Additional factors, for instance, cigarette smoking, progressed fatherly age, infections and inflammation of genital tract, drugs, hormones, raised scrotal temperature and varicocele, and intra-testicular components, for example, adjusted spermatozoal chromatin bundling, exorbitant reactive oxygen species (ROS) in seminal discharge and pre-ejaculatory abortive apoptosis subsidize to aetiology of spermatozoal chromatin defect.[2]

Varicocele is abnormally convoluted and enlarged spermatic veins in pampiniform rete having receding heme flux in internal spermatic vein. Varicocele is the commonest cause of sub-fertility among males, seen in 15% of healthy fertile male and 40% in sub-fertile men. Varicocele leads to defective spermatogenesis due to increased level of oxidative stress among sub-fertile males.[3]

Under aerobic conditions, human spermatozoa can produce ROS. Abnormal spermatozoa or seminal leukocytes are the main sources of excess ROS production endogenously. The loss of counterbalance among ROS generation and antioxidants defensive action to detoxify ROS by neutralization and removal leads to oxidative stress (OS). Relatively 25% of the subfertile males present with increased ROS levels and decreased antioxidant capacity of semen.[4]

Antioxidants are present in dietary substances and are also available as supplements. Both enzymatic and low molecular weight non-enzymatic form of anti-oxidants such as flavonoids are present in seminal plasma. Anti-oxidants prevent DNA fragmentation and damage. Standard antioxidants in semen incorporate vitamin C, vitamin E, glutathione, superoxide dismutase, and thioredoxin. These antioxidants kill free radical movement and shield sperm from ROS. Sub-fertile men have reduced anti-oxidant levels of semen and large amounts of ROS distinguished from fertile men, proposing an association between total antioxidant

capacity and male infertility. Among anti-oxidants, our focus of research was Quercetin.[5]

Quercetin is world's most broadly utilized dietary flavonoid. It is a reddish pigment found in apple, tea, vegetables, onion, and red wine. Quercetin is a strong anti-oxidant having cytoprotective properties in repressing oxidative damage and apoptosis caused by ROS and other forms of free radicals.[6]

Quercetin has been reported to mitigate effects of heavy metal toxicity on male reproduction. By scavenging free radicals Quercetin protects against reperfusion ischemic tissue injury. The role of quercetin as defensive agent has been reported on laboratory male animals' reproductive system and *in vitro* investigations on human semen samples so far.[7] Reproductive function in man has been emphasized to be conserved by quercetin. Quercetin has been demonstrated to enhance the functional and morphological integrity of the male reproductive system, as evidenced by increased sperm count, heightened tubular epithelial cells, augmented proliferative capacity and enhanced antioxidant enzyme activity and diminished levels of oxidative stress-related compounds. In rats, the higher concentration of Quercetin is found in testes and lungs and lowest in brain among all organs.[8] There are numerous reported approaches for the apprehension of quercetin quantitatively. The most common techniques for the quantifiable revelation of quercetin are High-Performance Liquid Chromatography (HPLC) and, High Performance Thin Layer Chromatography (HPTLC). The preferred method for the immediate determination of quercetin in food, plant extracts, body fluids and pharmaceutical products is HPLC.[9]

Despite the growing body of research on antioxidants and male fertility, limited studies specifically address the role of quercetin as a biomarker for oxidative stress. Recent advances in fertility research emphasize the importance of antioxidant supplementation, but their direct correlations with seminal parameters remain under explored. This study aims to bridge this gap by assessing the association of quercetin levels with male sub-fertility.

MATERIAL AND METHODS

The study was approved by the Ethics Committee of Imran Idrees Teaching Hospital, Sialkot, Pakistan. Subjects in the study provided written informed permission after being explained the overall purpose and process of the investigation. The sample size of 44 was calculated using OpenEpi software at a 95% confidence interval, with an effect size adequate for detecting significant differences in quercetin levels. The study population was divided into three categories, healthy fertile males as the control group (n=11), sub-fertile men without varicocele (n=22) and sub-fertile men with varicocele (n=11). Healthy samples were taken from general population and volunteers who were married males having children or their wives were pregnant at the time of study, and the sub-fertile group was categorized after obtaining a detailed history, clinical examination, and investigations of men married for more than one year going to the Infertility Clinic of the Hospital for their fertility assessment and therapy for lack of conception. Men with azoospermia and female factors responsible for sub-fertility or infertility were excluded from our study. Semen samples were collected in the vicinity of lab after an abstinence period of 3–4 days by masturbation in a sterile environment.

Sperm concentration, percentage motility, and sperm morphology were identified after liquefaction at 37 °C according to WHO criteria 2010 and sperm morphology by Kruger's strict criteria. Reliability of HPLC was ensured through calibration with standards from Sigma-Aldrich and validation using spiked semen samples with known quercetin concentrations. Reproducibility and accuracy were assessed with an intra-assay coefficient of variation of <5%.

Test sample (300 µL) was taken in a screwed test tube and 2 ml of 1:1 water and chloroform mixture were added. Then it was sonicated for 5 minutes with shaking. Chloroform layer was extracted in a separate screwed test tube. Aqueous layer was extracted twice with 1 ml portions of chloroform. Organic layers were combined and evaporated. Residue was dissolved in 0.5 ml ethanol and 50 µL of this solution was used for quercetin quantification via HPLC.

Hitachi Primaide HPLC system was used for quantification of quercetin in semen samples at a wavelength of 370 nm. HPLC/UV analysis were performed on a HITACHI Primaide Organizer System (Tokyo, Japan).

Data were entered and analysed on SPSS-21. Mean±SEM were used to represent data. One way ANOVA was applied, and $p<0.05$ was considered statistically significant.

RESULTS

Fertile men had an average age of 29.27±1.83 years, whereas the sub-fertile group without a varicocele had a mean age of 32.73±1.29 years and the sub-fertile varicocele group had a mean age of 34.64±2.24 years.

Table-1 shows a significant ($p<0.001$) increase in sperm count of healthy controls as compared to subjects with or without varicocele. The percentage of spermatozoal motility shows a significant ($p<0.001$) decrease in subjects with or without varicocele as compared to healthy fertile controls. In accordance to the Tygerberg strict criteria, mean percentage normal morphology of spermatozoa was non-significant ($p>0.05$) in varicocele-positive and varicocele-negative men in comparison to healthy controls.

Table-1: Seminal parameters according to WHO 2010 Criteria (Mean±SEM)

Sperm Parameters	Controls (n=11)	Sub-fertile males without varicocele (n=22)	Sub-fertile males with varicocele (n=11)	*p*
Semen volume (mL)	3.01±0.31	2.66±0.11	2.36±0.24	0.140
Sperm count (million/mL)	80.09±5.40	23.55±3.17	14.00±4.02	0.000*
Sperm motility (%)	77.73±3.72	34.77±6.51	30.18±7.13	0.000*
Normal sperm morphology (%)	15.82±1.63	14.59±2.06	10.27±2.64	0.276
Leukocyte count (million/mL)	0.53±0.04	1.42±0.49	0.40±0.19	0.177

*Highly significant using ANOVA

Figure-1 shows low levels of anti-oxidant Quercetin ($p<0.01$) in sub-fertile groups with or without varicocele as compared to healthy fertile controls.

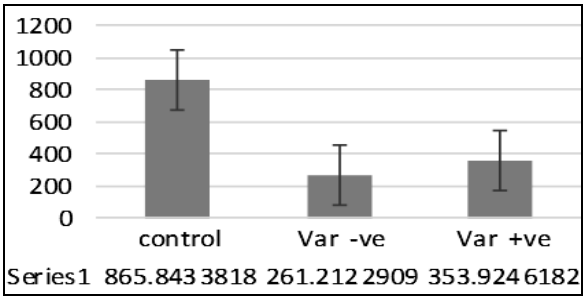

Figure-1: Levels of quercetin in control and subfertile men (µg/mL)

Var -ve: subfertile without varicocele, Var +ve: subfertile with varicocele

DISCUSSION

This study aimed to evaluate seminal parameters and quercetin levels in fertile and sub-fertile males, with a focus on the impact of varicocele. The results indicated significant differences in sperm count, motility, and quercetin levels between fertile and sub-fertile males with or without varicocele.

The observed reduction in sperm count and motility in sub-fertile males with varicocele aligns with findings from several studies. A study by El-Khawagah *et al*[10] demonstrated that quercetin supplementation in semen extenders significantly improved sperm motility and viability in buffalo bulls after cryopreservation. Our study found that quercetin levels were significantly lower in subfertile groups, suggesting a potential deficiency in natural antioxidant defences, which could exacerbate oxidative stress and further impair sperm function.

In our study, sub-fertile males without varicocele showed a significant reduction in total sperm count and motility compared to healthy controls. This finding is consistent with the work of Avdatek *et al*[11] which reported that oxidative stress markers are elevated in infertile males, leading to impaired sperm quality. Our findings reinforce the notion that oxidative stress plays a crucial role in male infertility, particularly in the presence of varicocele, where the stress levels are likely to be higher due to impaired blood flow and increased testicular temperature.

The non-significant differences in sperm morphology between the groups observed in our study contrast with the findings of other studies, such as that by Silva *et al*[12] who reported significant improvements in sperm morphology with antioxidant supplementation, including quercetin. This discrepancy might be attributed to differences in study populations, methodologies, or the specific antioxidants evaluated.

Quercetin levels were significantly lower in subfertile groups compared to healthy controls, a finding that is consistent with other studies emphasizing the role of antioxidants in preserving sperm quality. Tvrda *et al*[13] highlighted the protective effects of quercetin against oxidative damage in bovine spermatozoa, particularly in maintaining mitochondrial function, which is crucial for sperm motility. This parallels our observation that reduced quercetin levels are associated with diminished sperm motility in sub-fertile males.

The findings of this study align with existing literature emphasizing the role of oxidative stress in male sub-fertility and the potential therapeutic value of antioxidants like quercetin. By demonstrating significantly reduced quercetin levels in sub-fertile men, particularly those with varicocele, our results highlight the need for targeted dietary or therapeutic interventions. Comparisons with broader studies on antioxidant profiles underscore the importance of integrating multiple biomarkers to better understand oxidative stress and its impact on fertility. Our study highlights significant differences in seminal parameters and quercetin levels between fertile and sub-fertile males, with varicocele exacerbating these differences. The results are consistent with the general research on the significance of oxidative stress and anti-oxidants in male fertility, notably the potential therapeutic effects of quercetin supplements in treating varicocele-related infertility. However, further study is needed to determine the best quercetin dosage techniques and to investigate its impact on other sperm characteristics, in other populations.

Antioxidant activity in biological systems is influenced by a complex interplay of various enzymatic and non-enzymatic factors. While quercetin was the primary focus of this study due to its established role as a potent dietary antioxidant, we recognize that it is only one component of the antioxidant defence system. Comprehensive assessment of antioxidant activity would ideally include a broader range of biomarkers, such as glutathione, superoxide dismutase, catalase, and total antioxidant capacity. These biomarkers could provide a more holistic view of the oxidative stress status and its implications on male fertility.

The decision to focus on quercetin was based on resource limitations and its emerging significance in fertility research. However, we acknowledge this as a limitation and propose that future studies expand their scope to include additional markers. By integrating these parameters, subsequent research can further elucidate the potential of antioxidants in mitigating oxidative damage and improving reproductive outcomes. One limitation of this study is the exclusion of body mass index (BMI) as a variable. BMI is a known factor influencing oxidative stress and could have provided additional insights into the relationship between oxidative stress, quercetin levels, and sperm quality. Including BMI as a parameter would have strengthened the findings and offered a more nuanced understanding of its role in male fertility. The cross-sectional design of this study limits its ability to establish causation between quercetin levels and sub-fertility-related oxidative stress. While the findings provide valuable insights into potential associations, a longitudinal or interventional study would better elucidate causal mechanisms. Future studies should overcome these limitations by involving bigger, more varied groups and studying the implications of quercetin supplementation over extended periods.

CONCLUSION

Sub-fertile males, particularly those with varicocele, exhibit significantly reduced sperm count, motility, and quercetin levels compared to fertile males. The findings highlight the role of oxidative stress in male infertility and suggest that lower quercetin levels may contribute to diminished sperm quality.

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QAF: Principal Investigator, concept and design of the work
SR: Drafting the work, write-up
FS: Analysis and interpretation of data
TN: Critical review
SI: Final approval of draft
RR: Literature review and write-up

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