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TO FIND THE EFFECT OF MICROSURGICAL VARICOCELECTOMY ON SPERM DNA FRAGMENTATION IN PATIENTS HAVING INFERTILITY

Original Research

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ABSTRACT

Background: Varicocele is a leading cause of male infertility, often contributing to impaired spermatogenesis and elevated sperm DNA fragmentation index (DFI) due to oxidative stress, hypoxia, and testicular hyperthermia. These molecular disruptions adversely affect semen quality, fertilization potential, and reproductive outcomes. Microsurgical varicocelectomy, recognized as the gold standard for varicocele treatment, targets venous reflux correction to improve both conventional semen parameters and sperm DNA integrity. This study aims to evaluate the efficacy of this surgical intervention in restoring male reproductive health.

Objective: To assess the impact of microsurgical varicocelectomy on semen parameters and sperm DNA fragmentation in infertile men with clinically palpable varicocele.

Methods: A prospective study was conducted at the Department of Urology, Civil Hospital, Bahawalpur, from January to June 2024. Twenty-seven infertile men aged 25–45 years with clinical varicocele were enrolled. Semen samples were collected preoperatively and six months postoperatively following WHO guidelines, and assessed for sperm count (million/mL), motility (%), morphology (% normal forms), and DFI (%) using sperm chromatin structure assay (SCSA). Stratified analyses were performed based on age, obesity, duration of infertility, varicocele grade, and smoking status. Statistical analysis was carried out using paired t-tests and subgroup comparisons with SPSS version 25.

Results: Post-surgery, mean sperm count significantly increased from 24.46 ± 12.04 to 38.01 ± 13.88 million/mL ($p<0.001$), motility improved from 45.40 ± 11.29% to 51.15 ± 10.46% ($p=0.040$), and morphology rose from 4.90 ± 1.74% to 6.40 ± 1.73% ($p=0.002$). DFI reduced markedly from 19.37 ± 3.07% to 9.46 ± 3.22% ($p<0.001$). Subgroup analyses indicated superior outcomes in younger, non-obese patients and those with shorter infertility durations.

Conclusion: Microsurgical varicocelectomy offers significant improvements in semen quality and sperm DNA integrity, making it a critical intervention in the management of varicocele-related male infertility.

Keywords: Infertility, Male; Microsurgery; Oxidative Stress; Sperm Count; Sperm DNA Fragmentation; Varicocele; Varicocelectomy.

INTRODUCTION

Varicocele, characterized by the pathological dilation of the pampiniform venous plexus within the scrotum, represents one of the most common potentially correctable causes of male infertility. Affecting approximately 15% of men in the general population and up to 40% of those presenting with infertility, varicocele has been strongly associated with impaired spermatogenesis and diminished semen quality (1,2). A central mechanism in varicocele-induced infertility is oxidative stress, which leads to elevated production of reactive oxygen species (ROS), resulting in sperm DNA fragmentation (SDF) and subsequent damage to sperm function. High levels of SDF have been correlated with reduced fertilization rates, poor embryo quality, increased miscarriage risk, and lower live birth outcomes, both in natural and assisted reproductive settings (3). Among various treatment modalities, microsurgical varicocelectomy has emerged as the gold standard for managing clinically significant varicocele due to its high success rate and minimal complications. This technique involves meticulous ligation of dilated veins while preserving critical structures such as the testicular artery and lymphatics, thus promoting the recovery of testicular function and improvement in semen parameters including sperm count, motility, and morphology (4,5). Notably, this procedure has also been linked to a significant reduction in SDF levels, which plays a vital role in enhancing fertility outcomes (6). Elevated SDF in men with varicocele is primarily attributed to testicular hyperthermia, hypoxia, and ROS accumulation. These factors compromise sperm chromatin integrity and ultimately reduce reproductive potential (7,8). By alleviating these underlying stressors, microsurgical varicocelectomy not only improves conventional semen parameters but also restores the molecular quality of sperm DNA. This improvement has been reflected in better outcomes in assisted reproductive technologies (ART), including higher fertilization rates, improved embryo quality, and increased chances of live birth (9).

Moreover, surgical correction has shown potential in enhancing spontaneous conception rates, potentially reducing the dependency on invasive ART procedures (10). Despite the well-established benefits, variability in treatment outcomes necessitates careful patient selection. Factors such as varicocele grade, baseline semen quality, SDF levels, and patient age can influence the degree of clinical improvement. The integration of SDF testing into routine male fertility evaluations allows for a more nuanced understanding of reproductive dysfunction and aids in tailoring individualized treatment plans (11,12). However, the extent to which microsurgical varicocelectomy improves SDF and semen quality across diverse patient profiles remains inadequately defined in current literature. In light of these considerations, the present study aims to evaluate the impact of microsurgical varicocelectomy on sperm DNA integrity and conventional semen parameters in infertile men with clinically palpable varicocele. By generating robust clinical evidence, this study seeks to support evidence-based decision-making and optimize fertility outcomes through personalized surgical intervention.

METHODS

This prospective study was conducted in the Department of Urology at Civil Hospital, Bahawalpur, over a six-month period from January 2024 to June 2024. Ethical approval was obtained from the institutional review board prior to initiation, and written informed consent was secured from all participants in accordance with the Declaration of Helsinki. A total of 27 male patients with clinical varicocele and a confirmed diagnosis of infertility were enrolled. Eligible participants were aged between 25 and 45 years, with a history of at least one year of unprotected intercourse without conception. Clinical diagnosis of varicocele was established through physical examination and confirmed by Doppler ultrasound. Exclusion criteria comprised men with hormonal imbalances, known genetic disorders, azoospermia, systemic illnesses, or those unwilling to undergo surgery or participate in follow-up. The sample size was calculated using a paired t-test model to detect changes in the sperm DNA fragmentation index (DFI), with an 80% power and a 5% level of significance. Based on previous literature, a minimum of 22 participants was required, and the sample size was increased to 27 to account for potential attrition (13-15). The primary outcome measure was the DFI, while secondary outcomes included conventional semen parameters such as sperm count, motility, and morphology. Additional baseline characteristics recorded for each participant included age, body mass index (BMI), duration of infertility, varicocele grade, and smoking status.

All patients underwent microsurgical varicocelectomy performed by a senior urologist using an operating microscope under general or regional anesthesia. The procedure involved high-precision ligation of dilated spermatic veins, ensuring preservation of the testicular artery, lymphatics, and vas deferens. Postoperatively, patients were given routine instructions and advised to abstain from ejaculation

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for at least three days prior to semen collection. Semen samples were obtained by masturbation following 3 to 5 days of abstinence, both preoperatively and six months postoperatively. Semen analysis adhered to World Health Organization (WHO) laboratory standards, assessing sperm concentration (millions/mL), motility (percentage of motile sperm), and morphology (percentage of normal forms). Sperm DNA fragmentation was evaluated using the sperm chromatin structure assay (SCSA), analyzed via flow cytometry, with DFI expressed as the percentage of sperm with fragmented DNA. Data analysis was carried out using SPSS version 25. Descriptive statistics were applied to characterize baseline and outcome variables. Continuous data were reported as mean ± standard deviation. Pre- and post-surgical differences in DFI and semen parameters were compared using paired t-tests. Further stratified analyses assessed the influence of age, BMI, infertility duration, varicocele grade, and smoking on surgical outcomes. A p-value of less than 0.05 was deemed statistically significant.

RESULTS

The study enrolled 27 male patients with a mean age of 34.93 ± 5.73 years and an average BMI of 24.45 ± 3.69 kg/m². The mean duration of infertility among the participants was 5.96 ± 2.98 years. Following microsurgical varicocelectomy, a statistically significant improvement was observed across all primary and secondary outcome measures. The mean sperm count increased from 24.46 ± 12.04 million/mL before surgery to 38.01 ± 13.88 million/mL after surgery ($p<0.001$). Sperm motility improved from a baseline of 45.40 ± 11.29% to 51.15 ± 10.46% postoperatively ($p=0.040$), and normal sperm morphology increased from 4.90 ± 1.74% to 6.40 ± 1.73% ($p=0.002$). Notably, the sperm DNA fragmentation index (DFI) showed a substantial decline, decreasing from 19.37 ± 3.07% to 9.46 ± 3.22% ($p<0.001$), indicating a significant improvement in sperm DNA integrity. Stratified analyses by age demonstrated that sperm count improvements were statistically significant in the 25–34 years ($p=0.032$) and 35–39 years ($p=0.039$) age groups, while no significant change was noted in the 40–44 years group ($p=0.118$). Interestingly, sperm motility improved significantly in the 40–44 years group ($p=0.033$), and sperm morphology improved significantly only in the youngest group ($p=0.007$). In all age groups, DFI reduction was significant ($p<0.05$), reflecting the procedure's consistent benefit across age brackets. When stratified by obesity status, non-obese participants (N=25) experienced significant improvements in sperm count ($p=0.001$), sperm morphology ($p=0.002$), and DFI ($p<0.001$). In contrast, improvements among obese patients (N=2) did not reach statistical significance, likely due to the small sample size. Sperm motility showed a non-significant trend toward improvement in both obese and non-obese individuals.

Duration of infertility also influenced outcomes. Sperm count improved significantly in both 1–5 years ($p=0.010$) and 6–10 years ($p=0.018$) duration groups. Sperm motility improved significantly only in the 1–5 years group ($p=0.039$), while morphology improvement was observed in the 6–10 years group ($p=0.017$). Both groups showed substantial DFI reductions ($p<0.001$). Varicocele grade stratification revealed that patients with grade I ($p=0.033$) and grade II ($p=0.020$) experienced significant improvements in sperm count, whereas those with grade III did not ($p=0.193$). Although sperm motility and morphology improved across all grades, these changes did not achieve statistical significance. However, DFI reduced significantly across all grades ($p<0.05$), reinforcing the universal benefit of the procedure on DNA integrity. Regarding smoking status, both smokers and non-smokers demonstrated significant increases in sperm count ($p=0.039$ and $p=0.002$, respectively). Sperm morphology significantly improved among smokers ($p=0.008$) but not among non-smokers ($p=0.116$). Sperm motility did not show significant change in either group. Importantly, both groups exhibited marked reductions in DFI ($p<0.001$), emphasizing the robustness of the intervention regardless of smoking behavior.

Table 1: Comparison of Pre- and Post-Surgery Semen Parameters and Sperm DNA Fragmentation Index (DFI)

Variable Pair	N	Pre-Mean ± SD	Post Mean ± SD	p-value
Sperm Count	27	24.46 ± 12.04	38.01 ± 13.88	0.000
Sperm Motility	27	45.40 ± 11.29	51.15 ± 10.46	0.040
Sperm Morphology	27	4.90 ± 1.74	6.40 ± 1.73	0.002
Sperm DNA Fragmentation Index (DFI)	27	19.37 ± 3.07	9.46 ± 3.22	0.000

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Table 2: Stratified Analysis of Pre- and Post-Surgery Semen Parameters and DNA Fragmentation Index (DFI) by Age Groups

Variable Pair	Age Group	N	Pre-Mean ± SD	Post Mean ± SD	p-value
Sperm Count	25-34 years	11	21.68 ± 12.76	35.71 ± 14.86	0.032
	35-39 years	10	27.15 ± 12.66	39.42 ± 11.58	0.039
	40-44 years	6	25.08 ± 10.37	39.90 ± 17.29	0.118
Sperm Motility	25-34 years	11	50.25 ± 7.58	51.31 ± 12.09	0.786
	35-39 years	10	46.12 ± 11.52	52.95 ± 8.84	0.210
	40-44 years	6	35.30 ± 11.60	47.87 ± 10.78	0.033
Sperm Morphology	25-34 years	11	4.70 ± 1.45	6.82 ± 1.78	0.007
	35-39 years	10	4.50 ± 2.09	5.70 ± 1.19	0.089
	40-44 years	6	5.93 ± 1.38	6.78 ± 2.27	0.537
Sperm DNA Fragmentation Index (DFI)	25-34 years	11	20.29 ± 3.30	8.98 ± 3.46	0.000
	35-39 years	10	19.38 ± 3.32	10.05 ± 3.00	0.000
	40-44 years	6	17.65 ± 1.40	9.33 ± 3.53	0.005

Table 3: Stratified Analysis of Pre- and Post-Surgery Semen Parameters and DNA Fragmentation Index (DFI) by Obesity Status

Variable Pair	Obesity Status	N	Pre-Mean ± SD	Post Mean ± SD	p-value
Sperm Count	Obese	2	24.05 ± 10.82	36.25 ± 1.20	0.324
	Non-obese	25	24.50 ± 12.33	38.16 ± 14.43	0.001
Sperm Motility	Obese	2	29.85 ± 3.32	47.60 ± 7.50	0.105
	Non-obese	25	46.64 ± 10.77	51.44 ± 10.72	0.098
Sperm Morphology	Obese	2	4.60 ± 1.13	4.75 ± 1.06	0.939
	Non-obese	25	4.92 ± 1.79	6.53 ± 1.72	0.002
Sperm DNA Fragmentation Index (DFI)	Obese	2	21.65 ± 3.61	5.50 ± 0.00	0.100
	Non-obese	25	19.18 ± 3.03	9.77 ± 3.13	0.000

Table 4: Stratified Analysis of Pre- and Post-Surgery Semen Parameters and DNA Fragmentation Index (DFI) by Duration of Infertility

Variable Pair	Duration Group	N	Pre-Mean ± SD	Post Mean ± SD	p-value
Sperm Count	1–5 Years	11	20.18 ± 10.54	36.39 ± 14.30	0.010
	6–10 Years	16	27.41 ± 12.43	39.13 ± 13.94	0.018
Sperm Motility	1–5 Years	11	42.20 ± 10.73	51.36 ± 10.18	0.039
	6–10 Years	16	47.59 ± 11.47	51.01 ± 10.97	0.361
Sperm Morphology	1–5 Years	11	4.77 ± 1.48	6.25 ± 1.98	0.071
	6–10 Years	16	4.99 ± 1.94	6.50 ± 1.60	0.017
Sperm DNA Fragmentation Index (DFI)	1–5 Years	11	19.84 ± 3.34	9.95 ± 3.29	0.000
	6–10 Years	16	19.04 ± 2.93	9.12 ± 3.23	0.000

Table 5: Stratified Analysis of Pre- and Post-Surgery Semen Parameters and DNA Fragmentation Index (DFI) by Varicocele Grade

Variable Pair	Varicocele Grade	N	Pre-Mean ± SD	Post Mean ± SD	p-value
Sperm Count	I	8	24.20 ± 10.06	40.35 ± 16.69	0.033
	II	14	24.39 ± 12.75	35.31 ± 12.16	0.020
	III	5	25.08 ± 15.35	41.86 ± 15.13	0.193
Sperm Motility	I	8	51.98 ± 8.59	52.31 ± 10.99	0.945
	II	14	43.98 ± 11.75	50.66 ± 9.73	0.105
	III	5	38.84 ± 10.18	50.66 ± 13.71	0.100
Sperm Morphology	I	8	4.95 ± 2.19	6.74 ± 1.45	0.077

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Variable Pair	Varicocele Grade	N	Pre-Mean ± SD	Post Mean ± SD	p-value
	II	14	4.98 ± 1.62	5.92 ± 1.69	0.089
	III	5	4.60 ± 1.61	7.18 ± 2.17	0.120
Sperm DNA Fragmentation Index (DFI)	I	8	18.60 ± 2.62	9.41 ± 3.45	0.000
	II	14	20.00 ± 3.17	9.36 ± 3.15	0.000
	III	5	18.82 ± 3.68	9.80 ± 3.75	0.012

Table 6: Stratified Analysis of Pre- and Post-Surgery Semen Parameters and DNA Fragmentation Index (DFI) by Smoking Status

Variable Pair	Smoking Status	N	Pre-Mean ± SD	Post Mean ± SD	p-value
Sperm Count	Yes	13	26.61 ± 12.37	40.02 ± 15.37	0.039
	No	14	22.47 ± 11.82	36.15 ± 12.63	0.002
Sperm Motility	Yes	13	44.38 ± 12.24	51.25 ± 7.78	0.102
	No	14	46.34 ± 10.72	51.06 ± 12.76	0.234
Sperm Morphology	Yes	13	4.82 ± 1.88	6.85 ± 1.71	0.008
	No	14	4.97 ± 1.66	5.97 ± 1.70	0.116
Sperm DNA Fragmentation Index (DFI)	Yes	13	19.70 ± 3.06	9.21 ± 2.89	0.000
	No	14	19.06 ± 3.16	9.69 ± 3.59	0.000

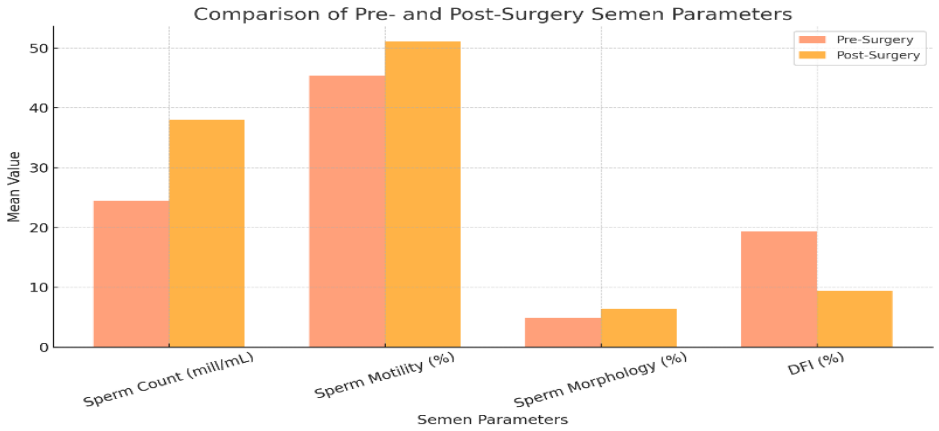

Figure 1 Comparison of Pre-and Post-Surgery Semen Parameters

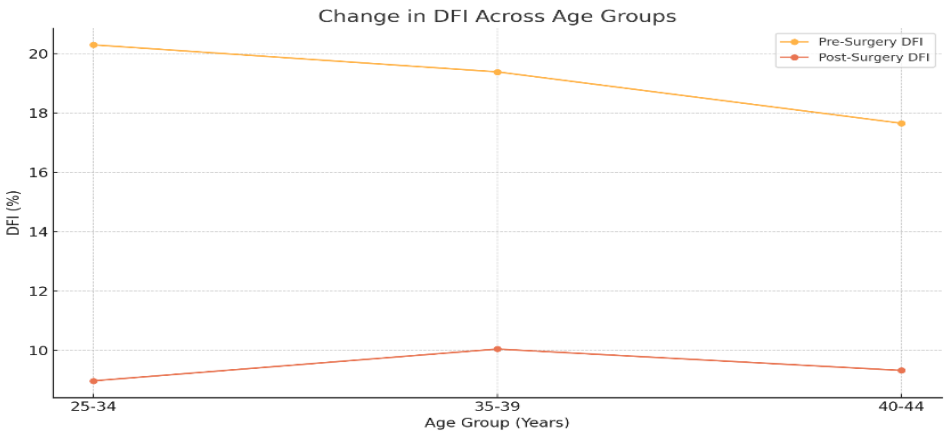

Figure 2 Change in DFI Across Age Groups

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DISCUSSION

The present study provides compelling evidence on the beneficial effects of microsurgical varicocelectomy in improving semen parameters and sperm DNA integrity among men with varicocele-associated infertility. These findings strongly support the therapeutic role of varicocelectomy in male fertility restoration, particularly by addressing oxidative stress-induced damage within the testicular microenvironment. The observed reduction in the sperm DNA fragmentation index (DFI) from 19.37% to 9.46% postoperatively represents a clinically meaningful improvement, consistent with earlier findings that underscore the role of surgical correction in reducing oxidative burden and restoring chromatin integrity (16-18). This reduction is particularly relevant, as elevated DFI is known to compromise fertilization potential, embryo development, and pregnancy outcomes. Improvements across all key semen parameters—sperm count, motility, and morphology—further substantiate the multifaceted impact of varicocelectomy. The significant postoperative increase in sperm count and enhancement in motility and morphology align with previously documented outcomes that attribute these improvements to normalization of testicular temperature, hormonal recovery, and improved blood flow to the testes (19-21). These parameters are critical in determining natural conception potential and enhancing outcomes in assisted reproductive technologies. Subgroup analyses offered further insight into the variability of surgical outcomes. Younger patients, particularly those under 40 years of age, exhibited more significant improvements in sperm count, suggesting a more favorable response to surgical intervention. The regenerative capacity of the testicular milieu may be age-dependent, highlighting the importance of early intervention in men presenting with clinical varicocele (22). Consistent with this, greater reductions in DFI were observed among younger age groups, reinforcing the link between age, oxidative stress resilience, and fertility potential.

Notably, patients with non-obese profiles demonstrated statistically significant gains across most semen parameters and DFI, while those with obesity did not exhibit similar levels of improvement. Although the limited sample size of obese patients in this study may have reduced the power to detect significant changes, these findings are reflective of broader literature indicating that obesity is associated with endocrine disruption, elevated oxidative stress, and impaired spermatogenesis. This highlights the need for individualized approaches in managing varicocele-associated infertility among men with metabolic comorbidities. The duration of infertility also appeared to influence outcomes. Men with shorter infertility durations (1–5 years) showed greater improvements in motility compared to those with longer durations (6–10 years), suggesting that prolonged varicocele exposure may result in irreversible testicular damage. Nevertheless, the consistent reduction in DFI across both groups supports the surgical benefit irrespective of infertility chronicity, at least with respect to DNA integrity restoration. Regarding varicocele grade, significant improvements in sperm count were more evident in patients with grade I and II varicocele, whereas those with grade III did not exhibit similar gains. This trend may reflect greater tissue compromise in advanced varicocele, where long-standing venous stasis and testicular hypoxia could limit the reversibility of damage. However, the consistent reduction in DFI across all varicocele grades indicates that the procedure offers meaningful molecular benefits even in more severe cases. Smoking status was also found to modulate outcomes. Although both smokers and non-smokers experienced significant reductions in DFI, non-smokers demonstrated slightly superior improvements in sperm count. These findings suggest that while varicocelectomy alleviates oxidative stress regardless of smoking behavior, lifestyle factors such as tobacco exposure may attenuate the magnitude of benefit.

The study's strengths lie in its prospective design, standardized surgical technique, and comprehensive analysis of both conventional and molecular semen parameters. The inclusion of stratified analyses based on age, obesity, infertility duration, varicocele grade, and smoking status adds depth to the interpretation of results and contributes to the growing body of evidence advocating for personalized approaches in male infertility management. Nevertheless, several limitations should be acknowledged. The relatively small sample size, particularly within subgroups such as obese individuals and those with grade III varicocele, limits the generalizability of stratified findings. The absence of data on pregnancy or live birth outcomes constrains the ability to link semen parameter improvements with clinical fertility success. Additionally, follow-up was limited to six months postoperatively; longer-term data could provide insights into the durability of the observed benefits. Future studies should incorporate larger, multicenter cohorts and extend follow-up periods to include reproductive endpoints such as spontaneous conception and ART success rates. Despite these limitations, the current study reinforces the clinical utility of microsurgical varicocelectomy as a cornerstone intervention for men with varicocele-associated infertility. Integration of sperm DNA fragmentation testing into routine infertility evaluations could enhance diagnostic precision and guide therapeutic decision-making. Furthermore, addressing modifiable factors such as obesity and smoking in conjunction with surgical intervention may further optimize outcomes. Exploration of adjunct therapies targeting oxidative stress and testicular microenvironment restoration could also offer synergistic benefits when combined with varicocelectomy.

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CONCLUSION

Microsurgical varicocelectomy has proven to be an effective intervention for improving both sperm DNA integrity and conventional semen parameters in men with varicocele-associated infertility. This study reinforces its clinical relevance, particularly among younger, non-obese individuals and those with a shorter history of infertility, where the therapeutic impact is most pronounced. By addressing underlying oxidative stress and enhancing reproductive potential, the procedure offers a valuable, evidence-based option within the broader framework of personalized infertility care, ultimately contributing to more favorable fertility outcomes.

AUTHOR CONTRIBUTION

Author	Contribution
Mir Abid Jan	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Khalil Ur Rehman*	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published

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