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In Vitro Assessment of Fibroin Silk Incorporated Glass Ionomer Cement for Solubility

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ABSTRACT

Background: Glass ionomer cement (GIC) is widely used in restorative dentistry due to its biocompatibility and fluoride-releasing properties. Incorporating silk fibroin into GIC has shown promise for enhancing its mechanical properties and reducing solubility, potentially improving its clinical performance.
Objective: This study aimed to compare the solubility of commercially available glass ionomer cement (control) with GIC modified with silk fibroin at 1%, 3%, and 5% by weight.
Materials and Methods: The study was conducted at Sardar Begum Dental College and Hospital – Peshawar. Specimens were prepared according to ISO guidelines using silk fibroin incorporated at 1%, 3%, and 5% concentrations by weight, mixed through a ball milling process. Solubility tests were performed by immersing specimens in distilled water at 37°C for 7 days. Statistical analyses were conducted using SPSS version 16.0, with one-way ANOVA and Tukey's HSD test used to determine significant differences ($p < 0.05$).
Results: Mean solubility values for the control group (Group A) were 0.000050 µg/mm³, while 1% silk fibroin (Group B) showed 0.000056 µg/mm³. The 3% silk fibroin group (Group C) demonstrated the lowest solubility at 0.000028 µg/mm³, while 5% silk fibroin (Group D) exhibited the highest solubility at 0.000084 µg/mm³. One-way ANOVA confirmed significant differences among the groups ($F = 32.159$, $p < 0.001$), with Tukey's HSD test revealing significant reductions in solubility in Group C compared to Groups A and B ($p < 0.05$).
Conclusion: Incorporating 3% silk fibroin significantly reduced the solubility of GIC, indicating its suitability for improved dental applications. Further studies should optimize fibroin concentrations for broader clinical use.
Keywords: Dental materials, Glass ionomer cement, Silk fibroin, Solubility.

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INTRODUCTION

One of a dentist's primary goals is to choose an appropriate restorative material to repair tooth structure lost due to caries, trauma, or other causes.[1] A suitable restorative material should effectively replace the missing tooth structure while meeting aesthetic requirements.[2] Glass-ionomer cements (GIC) combine the benefits of both silicate and polycarboxylate cements, making them one of the most extensively utilized aesthetic materials in modern dentistry.[3] They could be used as direct permanent filling materials, and also for lining and cementing. It is not only biocompatible and bioactive, but it also has the capacity to bond to wet tooth structure, such as enamel or dentine, making it a one-of-a-kind substance.[4]

Despite its benefits, this material's use is limited to areas without high chewing forces due to its low strength, fracture, toughness, abrasion resistance, and susceptibility to moisture contamination during setting.[2] Many attempts to enhance the physicomechanical properties of GIC have been made in the past attempts. Reinforcing GIC with hollow glass fibres, nano clay, short glass fibres, and zirconia revealed that the reinforced glass ionomer outperformed the conventional glass ionomer.[5] Solubility in oral fluids, has a significant impact on the restoration's longevity. A good material should be able to withstand dissolution over time.[6] A dental restorative solubility directly influences its longevity and potential interaction with biological tissues.[7]

Silk is a fibrous protein polymer found in nature with remarkable mechanical properties and biological compatibility. Fibroin is produced copiously by the caterpillar's silk gland as two polypeptide chains connected by a disulfide bridge.[8] Fibroin fibers have a unique combination of properties, such as strength, toughness, thermal stability and biocompatibility making them one of the most remarkable natural protein fibers, surpassing a variety of natural as well as synthetic fibers.[9] Mina et al. studied the mechanical qualities of silk incorporated glass ionomer cement, concluding that the silk fiber reinforcement led to a substantial improvement in properties of the typical GIC restorative material.[10,11] β sheet in silk fibroin makes it water-insoluble.[12,13]

This research addresses the need to improve the durability of glass-ionomer cement (GIC) used in dental restorations by incorporating silk fibroin. GIC, despite its biocompatibility, often exhibits weaknesses such as low mechanical strength and susceptibility to solubility in oral fluids. Previous studies have explored various

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reinforcements like nanoparticles and fibers to enhance GIC's physical properties. Silk fibroin, recognized for its robust mechanical characteristics and biocompatibility, presents a promising avenue for strengthening GIC. This study investigates the effects of incorporating silk fibroin at concentrations of 1%, 3%, and 5% on reducing GIC's solubility, aiming to prolong the lifespan of dental restorations and improve patient outcomes.

The significance of this research lies in addressing a critical gap in optimizing glass ionomer cement with reinforcing agents. While existing studies have demonstrated the effectiveness of nanoparticles and fibers in improving GIC's mechanical properties, silk fibroin remains underexplored despite its promising characteristics, such as high strength and biocompatibility. This study builds upon previous evidence suggesting that silk fibroin can reduce GIC solubility and enhance its performance. By systematically evaluating different concentrations of silk fibroin incorporation, the research seeks to establish its potential as a reinforcement strategy tailored to dental applications. The hook lies in its novelty: leveraging a biomaterial like silk fibroin to meet both functional and biocompatibility demands in restorative dentistry. Ultimately, this research contributes to filling the gap by providing concrete data on the optimal use of silk fibroin in GIC, paving the way for more durable and reliable dental restorations and advancing material innovation in clinical dentistry.

This study adheres to ISO standards by utilizing distilled water as the medium to assess GIC's solubility, ensuring alignment with established dental research practices. By examining how silk fibroin influences GIC's solubility under these conditions, this research aims to provide valuable insights into its potential as a dental restorative material. The findings could pave the way for future clinical applications, offering dentists and patients alike a more dependable and longer-lasting option for dental treatments. Therefore, this study investigated the solubility of commercially available glass ionomer cement (control) and glass ionomer cement incorporated with silk fibroin at concentrations of 1%, 3%, and 5% by weight

MATERIALS AND METHODS

This experimental research adopted a randomized controlled trial design conducted at Sardar Begum Dental College and Hospital – Peshawar. where specimen fabrication and solubility tests were performed. The study was conducted following ethical principles and received approval from the Ethical Review Committee of Sardar Begum Dental College and Hospital, Peshawar, on 14th November 2019 under Diary Number GU/Ethical Committee/2019. The materials and equipment used are comprehensively listed in Table 1 Specimens meeting uniform size criteria and free from imperfections such as air bubbles, voids, cracks, and visible porosities were included. Specimens with defects or those failing to meet size requirements, as measured by a digital vernier caliper, were excluded, along with any that experienced fabrication errors or contamination.

Operational Definition: The difference in the weight of the specimens before and after a period of 1,2,3,4,5,6,7th day after it has been immersed in distilled water for seven days. The results will be recorded in micrograms per cubic millimeter.[7]

Table 01. Materials and Equipment Used in the Incorporation of Silk Fibroin into Glass Ionomer Cement

S. No.	Materials	Chemistry	Suppliers
1	Fuji II Conventional glass ionomer (GC,Tokyo, Japan)	Fluoroaluminosilicate glass powder and a liquid (acrylic/maleic copolymer, polyacid, water)	Standard suppliers, Peshawar
2	Silk fibroin powder (Sigma-Aldrich)	Glycine-Alanine-Glycine-Alanine-Glycine-Serine	Standard suppliers, Peshawar
3	Distilled Water	Manahil Enterprises	Shah Dental Supply, Khyber Bazar Peshawar.
4	Digital Balance	Sartorius	NA
5	Laboratory Electro thermal Thermostatic Incubator	China	NA

The sample size for this study was determined through power analysis using statistical software, aiming to achieve 80% power at a significance level of 0.05. Based on anticipated effect sizes from preliminary studies,[12] a total of 40 specimens were required, with 10 specimens allocated to each of the four groups: Control group (A) and Experimental groups (B, C, D) with different concentrations of silk fibroin as shown in the figure 01.

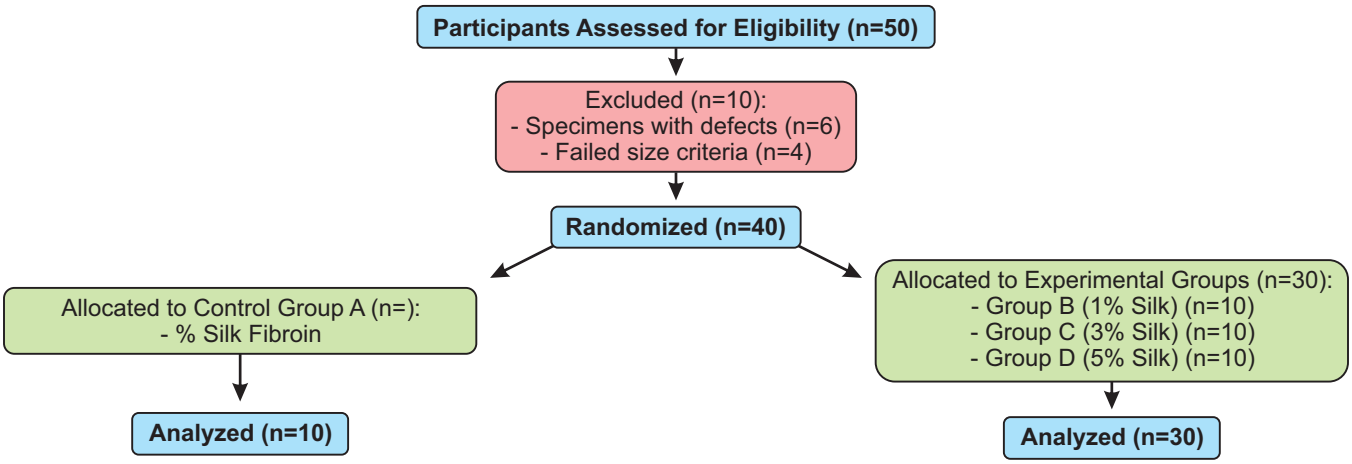


Figure 01. CONSORT Flow Chart

Measurements

For testing the solubility, stainless steel mould with an inner core diameter of 15mm and thickness of 2mm was constructed locally according to the ISO Guidelines Specification No. 4049: 2009 (e). Sartorius Analytical Balance of 10-4 (0.0001) precision was used for weighing the silk fibroin powder and glass ionomer powder for proportioning (Table 02).

Table 02. Composition of Glass Ionomer Cement (GIC) with Silk Fibroin

Groups	COMPOSITION (in grams) OF GIC and SILK fibroin.	Wt % of SILK FIBROIN
Control Group A	10 g of GIC	0
Experimental Group B	0.1 g silk + 9.9g GIC	1
Experimental Group C	0.3 g silk + 9.7g GIC	3
Experimental Group D	0.5 g silk +9.5g GIC	5

Silk fibroin powder was incorporated into GIC powder in 1%, 3%, and 5% by weight and was mixed uniformly with the help of a ball milling machine. The weighed amounts of glass ionomer cement and silk fibroin powders were mixed at a vibration frequency of 20 s^{-1} for 10 minutes. The ball-to-powder weight ratio was maintained (60 gram powders and 10 gram balls).[14] Separating media (Petroleum Jelly) was applied on the inside surfaces of the stainless steel split mould and placed on a glass slide. Mixture preparation adhered to the manufacturer's guidelines on the mixing pad. The homogenous cement was poured in the metallic mould, overfilled to avoid air inclusion and covered by another glass slide on top of it. After that, the mould was loaded with a 453.6g Gilmore needle for roughly 30 seconds to extrude the surplus material and create a uniformly flat specimen surface.

The glass slides were unloaded from the mould, and the specimens were removed once the material had fully set. The excess material along the edges was trimmed using a #15 scalpel blade (Bard-Parker, NJ, USA). This procedure was repeated for each specimen in every group. Immediately after removal from the mould, each specimen was visually inspected. Only specimens that were uniform in size, as measured with a digital vernier caliper, and free of voids were included in the study. Specimens exhibiting imperfections such as air bubbles, voids, cracks, visible porosities, or fractures were excluded from the study.

For 24 hours, all selected specimens were stored in the dry vacuum-desiccating chamber at 37°C. This led to complete polymerization and specimen dehydration, allowing for the calculation of solubility from a stable baseline, and then weighed to an accuracy of 0.1 mg using an analytic balance to get an initial mass m^1 value. The specimens were placed in glass vials containing 20ml of distilled water, wrapped in aluminium foil to keep out light, and incubated for 7 days at 37°C. After being gently wiped with absorbent paper for seven days, the specimens were reweighed and dehydrated in an air oven at 37°C for 24 hours before being reweighed; this measured mass was referred to as m^3.

The difference between each sample's original and final mass was used to calculate material loss (solubility). ($m^1 - m^3$). The value for solubility was computed using the equation:

$$S = \frac{m1-m3}{v}$$

Where; m^1 is the post-dehydration specimen mass before immersion in water. m^3 is the Specimen mass following seven-day immersion and desiccation. V is the volume of the specimen after it has been dehydrated but before it has been immersed in water. The volume of the specimen was calculated using the formula: $V=\pi r^2 h$ Where; r = D/2 and 'h' is the thickness of the specimen. A Digital Vernier calliper was used to measure the thickness of the specimen at four points. The values of solubility were measured in micrograms per cubic millimeter.

Statistical Methods

The data was analyzed using SPSS version 16.0. To assess the normality of the data, we performed the Shapiro-Wilk test. The Shapiro-Wilk test results indicate that all groups have p-values greater than 0.05, suggesting that the data does not significantly deviate from a normal distribution (Table 03). Thus, the assumption of normality is satisfied, and it is appropriate to proceed with the one-way ANOVA. One-way ANOVA was performed to compare group means and standard deviations. Post-hoc Tukey's HSD test was used for pairwise comparisons between groups at a 95% confidence level (p-value < 0.05).

Table 03. Shapiro-Wilk Test for Normality

Group	Shapiro-Wilk Statistic	Sample Size (n)	P-Value
Control Group A	0.972	10	0.832
Experimental Group B	0.959	10	0.716
Experimental Group C	0.941	10	0.588
Experimental Group D	0.924	10	0.462

RESULTS

Descriptive Data

This study investigated the solubility of commercially available glass ionomer cement (Control) and glass ionomer cement incorporated with silk fibroin at concentrations of 1%, 3%, and 5% by weight. Table 04 presents the descriptive analysis of solubility values for four groups, each with 10 specimens. The Control group A exhibited a mean solubility of 0.00004980 ± 0.000011641 µg/mm³. Experimental group B, with 1% silk fibroin, showed a mean solubility of 0.00005600 ± 0.000018667 µg/mm³. Experimental group C, with 3% silk fibroin, had the lowest mean solubility of 0.00002800 ± 0.000000000 µg/mm³. Experimental group D, with 5% silk fibroin, exhibited the highest mean solubility of 0.00008410 ± 0.000013437 µg/mm³. The overall mean solubility for all specimens combined was 0.00005447 ± 0.000023755 µg/mm³. The p-value from the one-way ANOVA was less than 0.001, indicating a statistically significant difference in solubility values among the groups.

Table 04. Descriptive Analysis of Solubility

Groups	N	Mean ± SD	P-Value*
Control group A	10	.00004980±.000011641	<0.001
Experimental Group B	10	.00005600±.000018667	
Experimental Group C	10	.00002800±.000000000	
Experimental Group D	10	.00008410±.000013437	
Total	40	.00005447±.000023755	

* Comparison of Solubility values of different groups using one way ANOVA

Table 05 provides the post hoc Tukey's HSD test results for multiple comparisons between groups. The comparison between the Control group and Group C (3% silk fibroin) shows a mean difference of 0.000021800 µg/mm³, which is statistically significant (p = 0.003). Similarly, the Control group and Group D (5% silk fibroin) have a significant mean difference of -0.000034300 µg/mm³ (p < 0.001). Group B (1% silk fibroin) shows no significant difference compared to the Control group (p = 0.706), but significant differences are noted when compared to Group C (mean difference = 0.000028000 µg/mm³, p < 0.001) and Group D (mean difference = -0.000028100 µg/mm³, p < 0.001). Additionally, Group C and Group D show a significant mean difference of -0.000056100 µg/mm³ (p < 0.001). Overall, significant differences in solubility were observed between most groups, particularly between higher concentrations of silk fibroin and the control.

Table 05. Post hoc Tukey's HSD for multiple comparisons between groups for solubility

(I) Groups	(J) Groups	Mean Difference (I-J)	P-value*
Group A (Control)	Group B	-.000006200	.706
	Group C	.000021800	.003
	Group D	-.000034300	<.001
Group B	Group C	.000028000	<.001
	Group D	-.000028100	<.001
Group C	Group D	-.000056100	<.001
Group D	Group A	.000034300	<.001

*The mean difference is significant at the 0.05 level.

The figure 02 shows the solubility values (µg/mm³) of specimens across four groups: Control, Group B, Group C, and Group D. Each bar represents the mean solubility for the corresponding specimen, with error bars indicating the standard deviation. The Control group consistently shows the lowest solubility values, ranging from 0.000027 to 0.000037µg/mm³. Group B has slightly higher solubility values, ranging from 0.000055 to 0.000065µg/mm³. Group C solubility values range from 0.000065 to 0.000084µg/mm³ showing a modest increase. Group D exhibits the highest solubility values, from 0.000084 to 0.000113µg/mm³, with specimen 9 reaching the maximum solubility of 0.000113µg/mm³. The increasing trend in solubility from the Control group to Group D suggests that the incorporation of silk fibroin into glass ionomer cement affects its solubility, with higher percentages leading to higher solubility values.

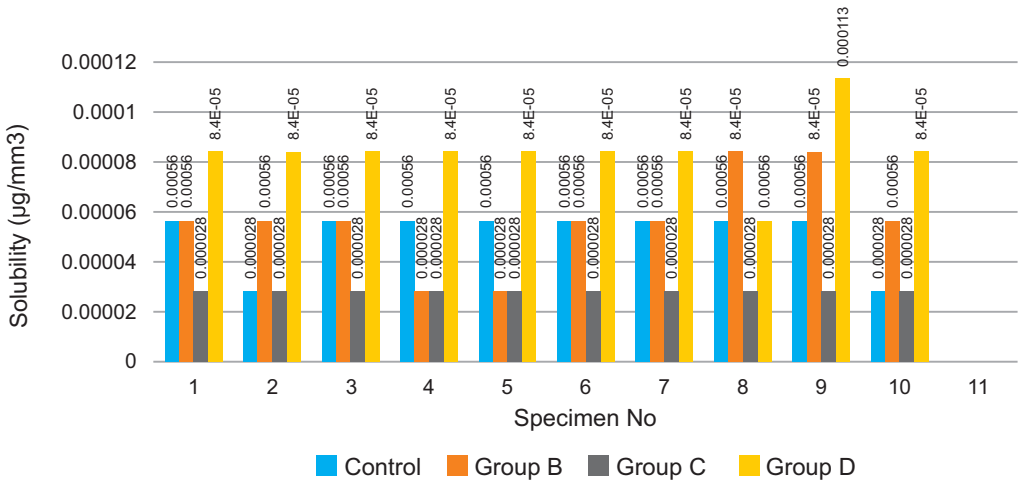


Figure 02. Solubility values after seven days (µg/mm3)

DISCUSSION

Glass ionomer cement being one of the bioactive materials, releases beneficial constituents e.g. fluoride which has an anti-cariogenic effect thus preventing further damage to the tooth structure. Because of its favorable release and aesthetic nature, glass ionomer cement is one of the most extensively used materials in dentistry. Despite their wide array of useful properties the high solubility and lack of strength are the limitations that prevent the clinician to make use of these materials to the fullest.[2]

Solubility is one of the most significant features of any dental material, since it determines the material's performance and longevity. If the released ingredient is harmful, it can have negative effect on the surrounding tissues, thus making the use of the material questionable. According to the literature, the glass ionomer cement has higher solubility values when it is constantly exposed to the oral environment, particularly acidic medium.[7]

Silk fibroin powder was mixed to glass ionomer powder in a ratio of 1, 3 and 5% by weight and the effects on solubility were studied. The solubility of the cement was calculated by weight analysis. Group B (with 1% silk incorporation) had a solubility value of 0.000056 g/mm3, which was not statistically significant when compared to control group A (p value > 0.05). The value of group C (with 3 percent silk fibroin incorporation) was 0.000028µg/mm3 which demonstrated a decrease in solubility as compared to conventional glass ionomer cement, indicating a statistically significant difference from group (p <0.05). The value of group D (with 5

percent silk fibroin incorporation) was 0.000084 µg/mm3, showing negative mean values thus statistically significant ($p<0.05$) in comparison to the values of control group A. These results thus partially reject the null hypothesis.

According to the results of the present study, it is obvious that a 1 percent incorporation of the silk fibroin into the GIC does not affect the solubility of the glass ionomer. However, the incorporation of 3 percent silk fibroin into the glass ionomer has reduced the cement's solubility. This could be attributed to the structural changes within the silk fibroin, making it more hydrophobic and less susceptible to water solubility. These structural changes were discussed by Jun et al.[15] and Asakura et al.[16] in their studies. They have found that the hydration of Bombyx mori silk fibroin results in a structural change from the random coil-shaped to the insoluble β-folded form due to an increase in hydrophobic contacts and hydrogen bond formation at a sufficient concentration of silk fibroin. The creation of the β sheet was linked by the breakdown of hydrogen bonds between water and peptides, which was preceded by the re-association of peptides, resulting in stronger hydrogen bonds and rendering the silk fibroin insoluble in water. The β folded forms have highly ordered (crystalline) areas within the polymeric structure. The reduction in the degradation of silk fibroin incorporated GIC can be attributed to the insoluble characteristic of silk fibroin in water which might be because it has a large number of intra- and intermolecular hydrogen bonds imparting it high crystallinity and intrinsic hydrophobicity,

Water in a polymer can be categorized into bound water and free water, as is widely known. It is thought that this bound water acts as a plasticizer.[17] Enough water molecules are required to allow for the movement of peptide chains, allowing for their alignment. The presence of water in the polymeric structure implies that the interactions between the water molecule and a tiny fraction of the glass ionomer's polar carboxylic groups are strong enough to prevent the water molecule from moving to other locations.[18]

The results of group D indicate a negative mean value which could indicate that this group suffered water absorption at a level that could have disguised the actual water solubility. The high hydrophilicity of glass ionomer-based materials explains this. The incorporation of a higher silk percentage seems to optimize this property. Another explanation of this behavior could be the swelling tendency of silk fibroin in water. A study conducted by Akpobome et al.[18] indicated that in various solvents including a neutral pH medium, the silk fibroin biofilm blend swelled. The silk fibroin blends swelled significantly in deionized water when the silk fibroin concentration was increased. A study also discussed in their study that silk Fibroin does not dissolve in water but rather it swells to 30.0 40.0% and attributed this property of silk fibroin to the structural design of the silk.[18] Silk fibroin has both amorphous and crystalline regions in its structure. The solvent that had been absorbed is retained by the polymer's amorphous portions. By increasing the content of the silk fibroin, the amorphous region in the structure of the molecule increases which in turn increases the swelling capability of the material. More research over a significant period of time is needed to determine the swelling and solubility pattern of silk fibroin-containing polymers for biological applications.

Limitation

The solubility of the cements was observed for a seven-day period. Distilled water was used instead of artificial saliva. Only the effects of silk fibroin on GIC at concentrations of 1,3 and 5% were investigated.

CONCLUSION

Incorporating silk fibroin into glass ionomer cement (GIC) at concentrations of 1%, 3%, and 5% by weight significantly influences its solubility. Group B, which contained 1% silk, exhibited solubility similar to that of the control group, whereas Group C, with 3% silk, showed a significant decrease in solubility. However, Group D with 5% silk exhibited increased solubility, potentially due to swelling effects. These findings underscore silk fibroin's potential as a reinforcement agent for enhancing GIC's durability in dental applications, paving the way for further research into optimizing its concentration for optimal clinical performance.

Ethical Consideration

This study was approved by Institutional Review Board of Sardar Begum Dental College and Hospital, Peshawar, Pakistan.

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Conflict of Interest

Authors declared no conflict of interest.

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DATA SHARING STATEMENT: The data that support the findings of this study are available on request from the corresponding author. The data is not publicly available due to privacy or ethical restrictions.

AUTHORS' CONTRIBUTION

The following authors fulfill authorship criteria as per ICMJE guidelines;

Irfan H, Imran M: Substantial contribution to conception and design, drafting the manuscript, final approval, agreed to be accountable for all the work.

Khan A, Ejaz M: Substantial contribution to conception and design, data acquisition, drafting the manuscript, final approval, agreed to be accountable for all the work.

Ahmasd SA: Analysis and interpretation of data, drafting the manuscript, final approval, agreed to be accountable for all the work.

Rehman K: Analysis and interpretation of data, critical revision, final approval, agreed to be accountable for all the work.

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