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Polymorphisms of the Gaurdian of genomes p53 And Functional Outcomes of Traumatic Brain Injury patients

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Abstract

One of the main causes of death is traumatic brain damage.The p53 gene's polymorphism and role as the genome's keeper have received the least attention in TBI.The study's goals were to assess the p53 gene's SNPs using bioinformatics techniques, identify patterns of Arg 72 Pro polymorphism in TBI patients compared to controls, and assess the relationship between p53 gene polymorphisms and the functional result of TBI. Using the NCBI accession IDs and their FASTA amino acid sequences, in silico analysis of SNPs of the p53 gene was carried out. Bioinformatics tools like SIFT, Polyphen-2, CADD score, MetaLR, and mutation assessor were used for the project. Using a string database, protein-protein interactions were evaluated.Using the PCR-RFLP approach, the effect of the Arginine variation of p53 was examined in a cohort of 58 people who had mild, moderate, and severe TBI. After three and six months following the accident, the neuropsychiatric results of the patients were evaluated using various questionnaires. nsSNPs of 15761 in total were filtered and analyzed. SIFT analysis of the SNPs in the p53 gene revealed that while 27% of the mutations were tolerated, 63% of them were detrimental. In the Polyphen-2 study, 64.20% of SNPs had detrimental mutations, compared to 35.42% of SNPs with benign mutations. 96.2% of the SNPs were found to have deleterious mutations according to the metaLR analysis, while 2.25% of them had tolerated mutations. According to CADD scores, the p53 gene had 7.42% detrimental and 91.77% tolerant changes.In terms of genotype allelic distribution, there was no discernible difference between patients and controls. Patients with the CC genotype had the highest mean GCS values. The proline-containing genotype (CC) had the highest GOSE score, which suggests a better outcome. Among all the outcome tools used, RFHUQ3 was the most sensitive (85.7%) and specific (90%) with a cut-off value of 6.5. The area under the curve (AUC) was the highest for RFHUQ at 3 months (0.958). The findings suggest that pathogenic p53 gene mutations and their interactions, as determined by bioinformatics software, may play a significant role in the etiology of TBI. The p53 gene polymorphism and functional outcome after TBI did not significantly correlate. Patients with the CC genotype (proline/proline) had fewer serious injuries, whereas GG-containing genotypes recovered to the fullest extent possible, which was confirmed by the fact that they spent the longest time in the hospital. When GOSE is the industry standard, RHFUQ was the most precise and sensitive technique to evaluate the functional outcome following TBI.

Keywords: p53, gene polymorphism, Traumatic Brain Injury, functional outcome, bioinformatics

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1. Introduction

Various feed additives contribute to the One of the most frequent causes of mortality and disability worldwide is traumatic brain injury (TBI). Studies have shown that apoptosis occurs in neurons and glia following TBI, which may be a factor in neurological dysfunction [1,2]. Reduced expression of survival-promoting proteins including Bcl-2 and extracellular signal has been associated with apoptotic cell death. Following TBI, up-regulated kinase and increased expression of proteins that cause death, such as Bax, c-Jun N-terminal kinase, p53, calpain, and caspases [1,3]. The p53 tumor suppressor factor controls DNA repair, cell cycle progression, apoptosis, and neural damage [4]. P53 is activated soon after a TBI, and blocking it is expected to offer neuroprotection [5,6]. An investigation was made into a functional polymorphism of the p53 gene in codon 72 that alters the properties of the produced protein in a group of 90 seriously head-injured patients admitted to an intensive care unit [7]. 78 patients with the Arg/Arg genotype had unfavorable results at the time of discharge. However, no significant difference between the patient groups with and without the Arg/Arg genotype was found 6 months later, indicating that p53 may only have a minimal impact on the long-term clinical outcome of people who have undergone TBI.

Studies that look at the connection between genes, their polymorphisms, and the results of severe traumatic brain injury are rare in literature. One such gene that hasn't gotten much attention in TBI is P53. Such a study will offer fresh perspectives on TBI therapy possibilities. Future clinical trials of treatments that specifically target these genes may be supported by the association between these genes, their polymorphisms, and the results of TBI. This will herald in a brand-new era of TBI treatment techniques utilizing genetic therapy. Gene polymorphism may serve as an early predictor of the consequences of a person's TBI. Only a small number of these studies are available, as far as we are aware. As a primary outcome measure for therapy in patients with traumatic brain injury, the Glasgow Outcome Scale (GOS) was used in the bulk of the study. GOS has a drawback, too, in that it lacks the sensitivity to spot minute but clinically important changes in TBI outcome. This is where the Glasgow Outcome Scale-Extended (GOSE) may be of assistance. The GOSE scale rates patient status into eight categories: death, vegetative state, severe upper and lower disability, moderate upper and lower disability, and satisfactory recovery, both upper and lower. The extended Glasgow outcome scale (GOSE) is a more sensitive and specific tool for evaluating the outcome than the Glasgow outcome scale, which was utilized as the gold standard tool in our study.

The Rivermead head injury follow-up questionnaire (RHFUQ) is a quick, simple, adequate, reliable, and valid assessment of outcome that may be used for injuries of all severity levels but is most useful following mild to severe head injuries. A new health-related quality-of-life (HRQoL) tool called the QOLIBRI-OS (Quality of Life after Brain Injury- Overall Scale) was created specifically for those who have had traumatic brain injury (TBI). It comprises an overall score as well as a six-domain HRQoL profile. Whenever a study is being undertaken to evaluate the significance of an SNP in disease, choosing which SNPs to include in the study is a difficult task. Using bioinformatics prediction methods, it may be feasible to distinguish between functional and neutral SNPs in such cases. With the aid of bioinformatics tools, the study aims to investigate the SNPs of the p53 gene as well as the functional outcomes of TBI in terms of GOSE, RHFUQ, and QOLIBRI-OS as well as their relationships to p53 gene polymorphism.

The study's goals were to:

1.conduct in-silico analysis of p53 SNPs utilizing bioinformatics techniques.

2. compare the p53 gene's patterns of Arg 72 Pro polymorphism in TBI patients to controls.

3.find out if there is a relationship between p53 gene polymorphisms and the functional outcome following traumatic brain injury as measured by the extended Glasgow outcome scale (GOSE), the Rivermead head injury service follow up questionnaire (RHFUQ), and the quality of life after brain injury (QOLIBRI-OS).

2. Materials and methods

Using the NCBI accession IDs and their FASTA amino acid sequences, in silico analysis of SNPs of the p53 gene was carried out. Bioinformatics tools like SIFT, Polyphen-2, CADD score, MetaLR, and mutation assessor were used for the project. Using a string database, protein-protein interactions were evaluated. The current cohort study was carried out in collaboration with the Department of Neurosurgery at KSHEMA in Mangalore, India, in the Central Research Laboratory's Molecular Division's Department of Biochemistry. The age range of 18 to 60 years was used to recruit TBI patients with mild, moderate, and severe injury after resuscitation and stabilization at admission. Before beginning the study, clearance from the university's ethical committee was obtained. Patients with TBI who also spinal cord injuries, neurological conditions, or cerebrovascular diseases had were not included.

Sample collection and evaluation:

After obtaining three mL of EDTA whole blood and signed informed consent from the patient or their first-degree relatives, genotyping was carried out. To isolate DNA, the salting-out method was employed. The DNA quality was confirmed using electrophoresis on a 0.8 percent agarose gel with ethidium bromide (0.5 g/ml) in TAE buffer. DNA quality and quantity were evaluated using the spectrophotometer (OD260/OD280 ratio).

Amplification and genotyping:

By using PCR-RFLP, the p53 gene was genotyped. Table 1 lists specifics on forward and reverse primers, restriction enzymes, and reaction conditions. Functional outcome after TBI was carried out using Outcome tools like Rivermead head injury service follow-up questionnaire (RHFUQ), quality of life after brain injury (QOLBRI-OS), and extended Glasgow outcome scale (GOSE). Outcome assessment was done at three months and six months following injury.

Statistical analysis:

Graphpad Instat version 3 was used to conduct the statistical analysis. Weinberg, Hardy Equilibrium (HWE) was utilized to analyze the allelic distribution, and the 2 test was applied to determine whether there was a relationship between the genetic polymorphism and the result. To assess functional outcomes between various allelic variations as well as to compare the scores at admission, discharge, 3 months, and 6 months, Kruskal Wallis was used, followed by the post hoc test and Dunn's test. Whitney Mann A U exam was used to compare the group's scores at three and six months. In order to compare the effectiveness of outcome instruments, receiver operative characteristic curves (ROCs) were built. The correlation between length of stay and GOSE functional scores, RHFUQ and QOLIBRI-OS was evaluated using Spearman's correlation coefficient.

3. Results and Discussions

15761 nsSNPs in total were filtered and analyzed. SIFT analysis of the SNPs in the p53 gene revealed that while 27% of the mutations were tolerated, 63% of them were detrimental. In the Polyphen-2 study, 64.20% of SNPs had detrimental mutations, compared to 35.42% of SNPs with benign mutations. 96.2% of the SNPs were found to have deleterious mutations according to the metaLR analysis, while 2.25% of them had tolerated mutations. According to CADD scores, the p53 gene had 7.42% detrimental and 91.77% tolerant changes. A total of 0%, 21.85%, 69.64%, and 6.16% of the mutations were classified as high, low, medium, and neutral using the mutation assessor tool. The outcomes of the effect prediction are shown in table 2. Fifty-eight TBI

cases with a mean age of 38.66± 12.2 (17,62) years and fifty-eight controls with the mean age of 34.3± 12.87 (21,60) years were the study participants. The ratio of males to females was 3:1 in cases and 4:1 in controls. The causes of injury were mainly road traffic accidents (RTA) (71.4%) and falls (26.19%), assault, and other (2.41%). Restricted Polymerase Chain Reaction and with the aid of 2% Agarose gel electrophoresis, digested fragments were seen and fragment length polymorphism was seen (Table 1). Undigested fragments (448 bp) are designated CC, 248 bp fragments are designated GG, and CG is identified by both 448 bp and 248 bp bands (Figure 3). The allelic distribution of genotypes, including homozygous dominant (GG) (wild), heterozygous (CG), and homozygous recessive (CC) (mutant) alleles, did not significantly differ between patients and controls (Table 3; chi-square values, 0.004 and 0.233, respectively; p>0.05).

The chi-square values for the association between the p53 polymorphism genotypes and the GCS at admission, GOSE, RHFUQ, and QOLBRI-OS at 3 months and 6 months were 0.432, 2.488, 1.102, 2.745, 0.002, and 1.430, respectively. The allelic distribution showed no statistically significant gender difference (chi-square = 2.05). Between the three p53 alleles, the GCS levels did not significantly change. However, patients with the CC genotype had the highest mean GCS values (Table 3). Although there was no statistically significant difference between the alleles at 3 months or 6 months on the extended Glasgow outcome scale (GOSE), the proline-containing genotype (CC) had the highest score, indicating a better result. At both 3 months and 6 months, patients with CC alleles had the lowest mean RHFUQ score, indicating a good recovery. Patients with p53 genotypes CG and GG, which contain arginine, had RHFUQ scores that were on the upper side, indicating a poor prognosis (Table 4). The QOLIBRI-OS mean score similarly displayed the highest values across CC. The mean score for QOLIBRI-OS also showed the highest values among CC carriers at 3 and 6 months suggesting good recovery compared to CG and GG carriers (Table 4) Mortality among study subjects was noted only among GG and CG carriers. Whereas, among CC there were no deaths.

Extended Glasgow Outcome Scores showed a significant improvement from 3 to 6 months among GG carriers (p=0.03). RHFUQ also showed a similar fashion of improvements with the p=0.05 among GG carriers. Whereas, QOLIBRI-OS showed significant improvement from 3 to 6 months among CC and GG carriers (p= 0.03 and 0.02 respectively) (Table 5). However, significant improvement was observed in all the TBI patients from 3 to 6 months irrespective of the gene variant. RHFUQ values were significantly lower compared to 3 months (p=0.03), GOSE value was significantly higher during 6 months follow up compared to 3 months (p=0.05). Similarly, QOLIBRI-OS values were significantly increased during 6 months (p=0.01). When taken account of 3 and 6 months of follow-up of all subjects without considering their genotype, it was noted that there were 3 deaths recorded making 6.82% of the population under study. Lower GOSE scores (2-5) were recorded in 34.09% and 21.95% during 3 and 6 months follow up respectively. A good prognosis (GOSE <5) was observed in 59.09% at 3 months and 78.08% at 6 months among the survivors (Table 6).

RHFUQ values were categorized as <10 and >10 ; <10 being considered good prognosis. About 70.7% showed a good prognosis at 3 months which increased to 80.49% at 6 months. Poor prognosis was noted in 29.26% of subjects in 3 months which was reduced to 19.51% during 6 months. Health-related Quality of life was assessed using QOLIBRI-OS for all the subjects. The total score is divided as < 18 and >18, the latter being considered a good-quality index. Only 12.2% and 9.76% of subjects had scores below 18 at 3 and 6 months respectively. 87.8% of subjects scored good quality index at 3 and 6 months which increased up to 90.24% at 6 months (Table 6). The average length of stay in hospital (LOS) for all subjects was a median value of 6.5 (4 -11.5) days (Figure4). Whereas for TBI survivors it was 6 (4 - 12.5) days and among diseased due to TBI average LOS was noted to be 7 (1, 10) days. Length of stay in the hospital was the highest for patients with GG 8.5 (5.5 -13.5) as compared to CG 6 (4 9) and CC 6.5 (3.5 18.3) in days. The extent of improvement was shown by 1.09,1.10 and 1.27 in CC, CG, and GG respectively at 6 months as compared to 3 months by GOSE. Improvement by RHFUQ was 2.04, 1.48, and 2.39 respectively in the above-mentioned alleles. The degree of improvements in QOLIBRI was the same in all three gene variants. However, LOS was not statistically significant (p=0.689) in various genotypes. The correlation of functional scores with the LOS in the hospital is as depicted in table 7. AUC for QOLBRI was 0.114 at 3 months with a cut-off of 26.5, with low sensitivity and specificity of 19% and 15% respectively.

Both LOS and GOSE scores and LOS and QOLIBRI-OS scores showed no connection. However, at 3 and 6 months following the injury, there was a significant association between LOS in days and RHFUQ scores. ROC examined the effectiveness of the outcome evaluation instruments in assessing patients' recovery from TBI. RHFUQ had the best sensitivity, specificity, and AUC at both 3 and 6 months (Figures 5 and 6). With a cut-off value of 6.5, the RFHUQ3 was the most sensitive (85.7%) and specific (90%) of all the outcome tools used. The RFHUQ had the largest area under the curve (AUC) at 3 months (0.958) (Figure 5). AUC for QOLBRI at 3 months was 0.114 with a cut-off of 26.5. RFHUQ was the best outcome assessment tool at 6 months as well with the highest sensitivity of 100% and specificity of 91% and a cut-off value of 7.5. AUC was 0.968 (Figure 6). AUC for QOLBRI at 6 months was also low, 0.119, with a cut-off of 24.5 with very low sensitivity and specificity of 25% and 10% respectively.

Discussion

The SIFT scale has values between 0 and 1. Depending on whether a single nucleotide polymorphism's SIFT score is less than or equal to 0.05, it is either considered harmful or tolerable. The optimal range for the median information is between 2.75 and 3.5. This allows for the evaluation of the prediction sequences' variety. Indicators that the forecast was based on closely similar sequences include a number greater than 3.25. The PolyPhen software also looked at the SNPs. A score of 0.0 to 0.15 indicates a benign mutation, 0.15 to 1.0 indicates potential harm, and 0.85 to 1.0 indicates certain, foreseeable injury. CADD Scores under 30 are thought to be "likely benign," whereas scores over 30 are thought to be "likely harmful''. The 0.1% of the most harmful potential substitutions in the human genome are projected to be those variants with scores > 30. In addition to the likelihood, which might be "neutral," "low," "medium," or

"high," MetaLR also displays the rank score, which ranges from 0 to 1, with higher scores suggesting changes that are more likely to have negative effects. More negative potential is indicated by higher scores on the mutation assessor scale, which ranges from 0 to 1. Different genotype expressions of p53 exist, including homozygotic Pro72Pro, heterozygotic Arg72Arg, and homozygotic Pro72Pro. They can be homozygous dominant (GG), heterozygous (CG), or homozygous recessive (CC) depending on whether proline or arginine is present. G and C both include alleles that code for arginine and proline, respectively.

Among the current investigation, the "C" allele predominated among controls. 'G' allele was predominated in those situations, though. However, there was no significant difference. observed and expected allele frequencies as per HWE results ($p>0.05$). Numerous genotype variants were found [8] as a result of variances in electrophoretic mobility. The outcome of TBI patients has been associated with the p53 SNP. The p53 protein's two variations, Arg/Arg and Arg/Pro, each have unique functional properties [9–12]. While the Arg72Pro form arrests the cell cycle in G1 and triggers p53-dependent DNA repair, the Arg72Arg form more potently promotes apoptosis [13–16]. 70% of South Africans and 23% of Western Europeans have the Pro72Pro allele of the Arg72Pro gene. The Pro72Pro allele appears to offer higher protection against sun-related disorders, at least according to the latitude gradient from Europe to Africa [17]. The few epidemiological studies that examined the relationship between the Arg72Pro genotype and skin cancer risk had varying results [18,19]. Patients with the CG and GG genotypes had lower moderate disabilities at three months and upper-moderate disabilities at six months, whereas those with the CC genotype had higher moderate disabilities at three months and lower excellent recovery at six months. A similar tendency in the recovery pattern was also revealed by the RHFUQ questionnaire, which contrasts patients' experiences before and after injury. A higher score indicates a poor recovery, and the opposite is true. Although not statistically significant, the highest score was seen in individuals with GG, suggesting that their chances of recovery were worse. At 3 months and 6 months, CC genotypes' minimum scores were recorded (table 3). RHFUQ was created to assess social and functional outcomes (at the level of disability), hence it was necessary to develop a brief, easy-to-use tool. The RHFUQ questionnaire is an effective clinical instrument for assessing the daily struggles and experiences of people with head injuries. It addresses disability and the effects of function loss or impairment. When ROC was constructed, it was discovered that the RHFUQ questionnaire was extremely effective and comparable to GOSE, with the maximum area under the curve at both 3 and 6 months (Figure 5 and Figure 6). The questionnaire also showed the highest sensitivity as well as specificity. It is the reality that the patient's personal and social circumstances, the accompanying injuries, the patient's attitude to the accident, any prior injuries, and the injury itself all have an impact on the impairment. The RHFUQ questionnaire just asks patients to describe the degree to which they believe that some of the most fundamental parts of their daily life have changed. It makes no attempt to separate these factors. The rating scale, as opposed to straightforward "yes/no" answers, broadens its scope to reflect the discrepancy in injury outcomes for patients, whose circumstances for injuries vary greatly. This questionnaire has performed on par with GOSE in terms of evaluating daily activities.

The Quality of Life After Brain Injury-Overall Score (QULBRI) measures patients' overall welfare; a score of 30 indicates a good or satisfied life, while a score of 6 indicates the least satisfaction. At both 3 and 6 months, CC genotypes have the highest QOLIBRI-OS scores (table 4). But when building ROC, the QOLBRI at 3 and 6 months displayed a poor area under the curve (Figures 5 and 6), as well as the lowest sensitivity and specificity when compared to other questionnaires. GG genotypes had the highest levels of recovery despite having the lowest outcome scores. Their longest length of stay (Figure 4) supports this observation.
Significant A longer length of hospital stay may be associated with patients who have poor outcomes, according to a positive connection between RHFUQ scores at 3 and 6 months and LOS (Table 6). Similar to the patient's state and psychological factors, this association is strong. We can infer that patient psychosocial recovery is also an important factor in overall recovery and that patient rehabilitation in this regard needs to be improved. Longer hospital stays, which were observed among those who scored higher on the RHFUQ, suggest that patients' psychosocial recovery is also an important factor in overall recovery in addition to recovery from the traumatic condition and recovery in terms of health-related queries. The ROC also supports this conclusion (Figure 5 and 6). TBI recovery varies depending on the patient's age, as well as the kind, where, and how much of an injury it was [20–22]. The variation in results is not entirely explained by established predictors. A prospective study by Martinez PL et al. involving 90 Caucasian patients who had suffered severe TBI and 100 healthy controls was published in 2005[7]. The study discovered no appreciable variations in the frequency of Arg72Pro polymorphisms from controls. The results of a TBI were evaluated at discharge and six months later using the Glasgow outcome scale. The Arg/Arg genotype was associated with 69 percent of unfavorable outcomes, according to the research. Additionally, it was found that Arg/Arg variants were 2.9 times more likely to have a subpar discharge result. Individuals with the Arg/Arg genotype and those with the Arg/Pro and Pro/Pro genotypes did not have statistically different hospital stays. The Arg/Arg polymorphism and mortality were not linked, according to the investigation [7]. There was a statistically significant genotype difference and a dismal prognosis six months after TBI. The Glasgow Outcome Scale at Discharge was used as the outcome assessment tool in another study by Martinez PL et al., which included 90 patients with severe TBI. The study found that 81.1% of the patients had a poor outcome and 18.9% of them had a favorable outcome. The findings unambiguously demonstrated that Arg/Arg polymorphism was a separate predictor of bad outcome, with the chance of a poor outcome being 3.55 times higher with Arg/Arg genotypes, which was consistent with their earlier data [23].

Table 1: PCR-RFLP Reaction Conditions for the genotyping of p53

SNP	Location (Base change)	Forward Primer Reverse Primer	PCR Program (35 cycles)	PCR Fragment length (Bp)	Restriction enzyme, Incubation temperature	Allele: RFLP fragment size
p53 Arg72Pro (rs1042522)	Promoter G>C Arg>Pro	5' CCTGAAAACAACG TTCTGGTAA 3' 5' GCATTGAAGCTCC ATGGAAG 3'	94°C, 5" 94°C,30', 55°C,30', 72°C,30' 72°C, 7"	448bp	BstUI, 37°C	248 bp

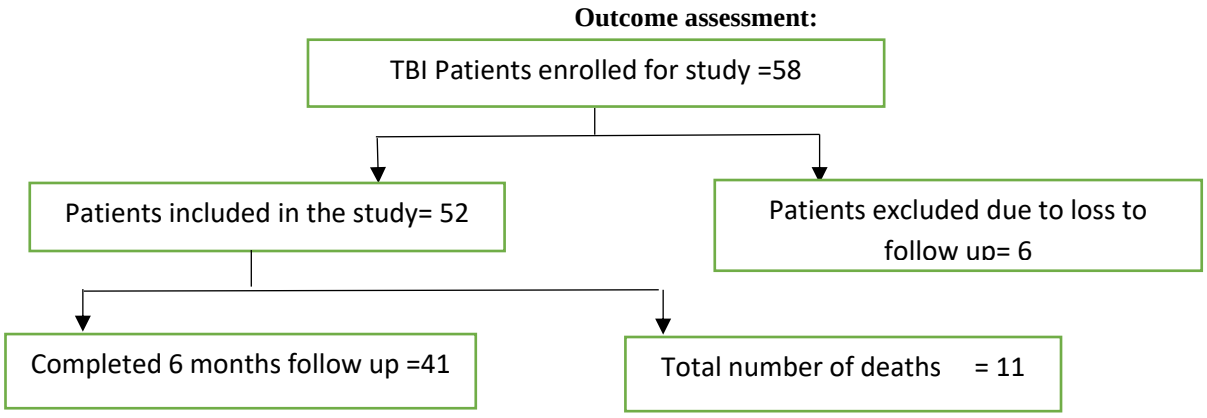

Figure 1: Flowchart depicting patients included in the study.

Table 2: Bioinformatics of p53 gene

SIFT		Polyphen-2		CADD		MetaLR		Mutation Assessor	
Deleterious low	163	Benign	5584	Deleterious	1170	Damaging	15163	High	0
Deleterious	9777							Low	3444
Tolerated low	180	Possibly damaging	10120	Benign	14465	Tolerated	355	Medium	10976
Tolerated	5466							Neutral	972

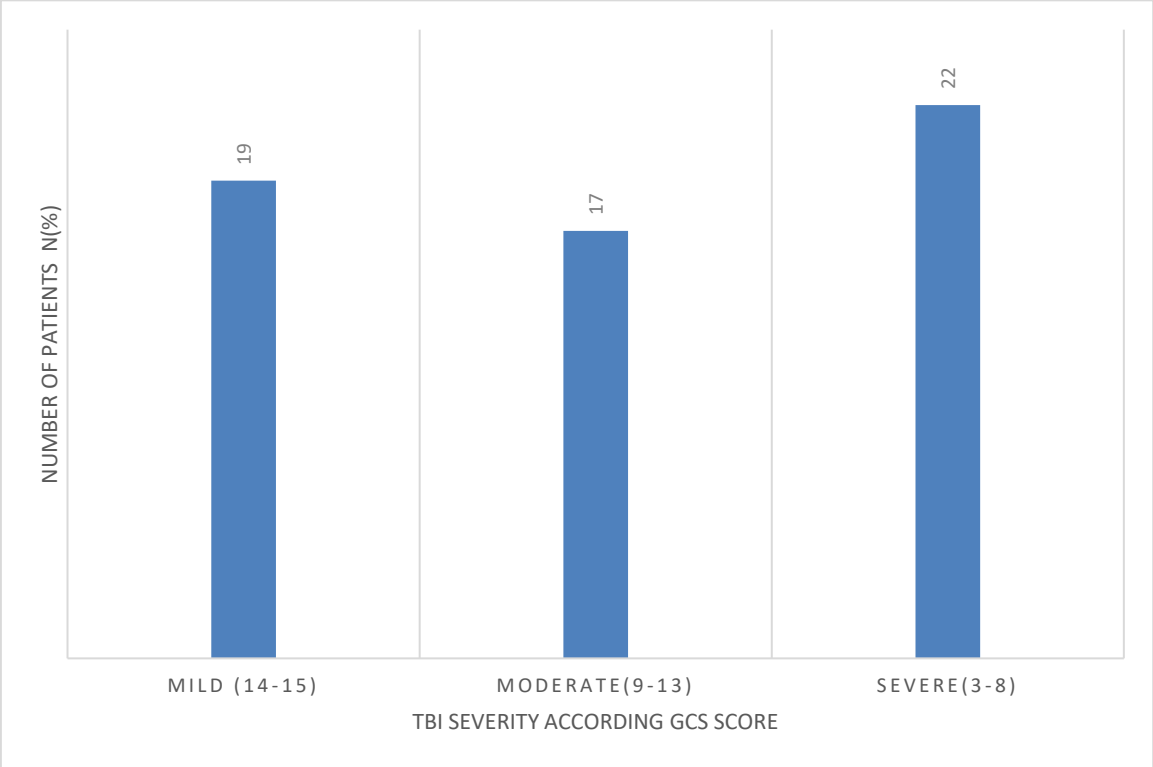

Figure 2: Showing grouping of TBI patients according to their GCS score on admission.

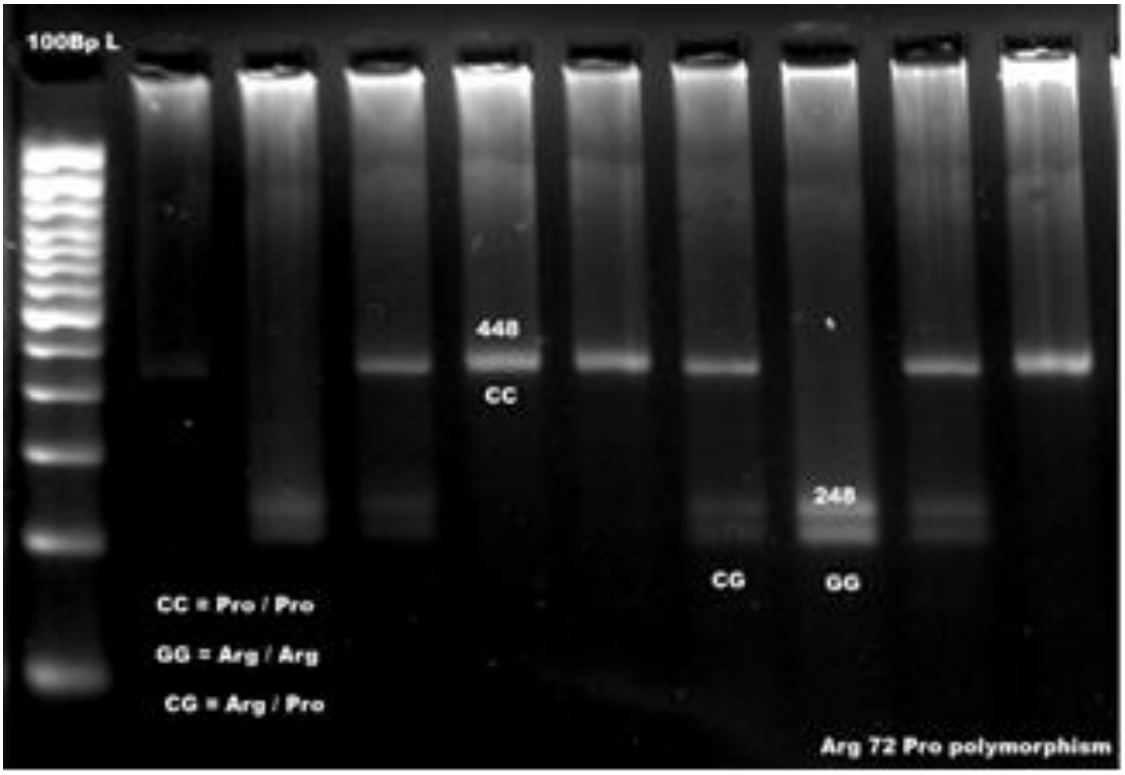

Figure 3: Agarose gel electrophoresis of RFLP digested p53 PCR with BstU1 restriction endonuclease.

Table 3: Comparison of Arg72Pro polymorphism between cases and controls.

Gene variant		Genotype frequency		χ^2 value
		Cases	Control	
CC	Observed	13	22	0.4741 (cases) (p=0.4741 q=0.525)
	Expected	13.03	21.12	
CG	Observed	29	26	
	Expected	28.92	27.75	0.2328 (Control) p=0.6034 q=0.393
GG	Observed	16	10	
	Expected	16.03	9.12	

Table 4: Comparison of Various Functional Outcome Scales in TBI patients with different genotypes of p53

p53 genotype	CC	CG	GG	p-value
GCS N=58	12.08± 4.14 (n=13)	10.4±4.5 (n=19)	9±4.08 (n=16)	0.14
GOSE- 3 months N=44	6.4±1.9 (3,8) (n=12)	5.68±1.77 (2,8) (n=19)	4.38±2.5 (1,8) (n=13)	0.06
GOSE-6 months N=41	7±1.7 (n=12)	6.26±1.69 (3,8) (n=19)	6.6±1.26 (4,8) (n=10)	0.26
RHFUQ- 3 Months N=41	2.5 (0.25 7) (n=12)	5 (0 14) (n=19)	10.5 (3.5 22) (n=19)	0.3
RHFUQ-6 Months N=41	0 (0 3.2) (n=12)	1 (0 9) (n=19)	3 (1 7.25) (n=19)	0.3
QOLIBRI-OS-3 Months N=41	24.9±8.33 (7,30) (n=12)	24.6±6.03 (6,30) (n=19)	24.3±3.43 (19,30) (n=10)	0.25
QOLIBRI-OS-6 Months N=41	27.7±5.85 (11,30) (n=12)	25.4±6.8 (6,30) (n=19)	27.6±1.96 (25,30) (n=10)	0.16

Test: Kruskal Wallis test; Values: Mean ±SD (min, max) (n) or Median (25% 75%)(n); p<0.05 significant*

Table 5: Improvement recorded within the same genotype with every follow-up.

		GOSE		RHFUQ		QOLIBRI-OS	
Genotype	Follow up	Score Mean ±SD(min, max) (n)	P value	Score Mean ±SD(min, max) (n)	P value	Score Mean ±SD(min, max) (n)	P value
CC	3 MONTHS	6.42±1.98 (3,8) (n=12)	0.41	2.5 (0.25 7) (n=12)	0.116	24.9±8.33 (7,30) (n=12)	0.036*
	6 MONTHS	7±1.71 (3,8) (n=12)		0 (0 3.2) (n=12)		27.7±5.85 (11,30) (n=12)	
CG	3 MONTHS	5.68±1.77 (2,8) (n=19)	0.25	5 (0 14) (n=19)	0.322	24.6±6.03 (6,30) (n=19)	0.258
	6 MONTHS	6.26±1.69 (3,8) (n=19)		1 (0 9) (n=19)		25.4±6.8 (6,30) (n=19)	
GG	3 MONTHS	4.38±2.5 (1,8) (n=13)	0.03*	10.5 (3.5 22) (n=10)	0.05*	24.3±3.43 (19,30) (n=10)	0.020*
	6 MONTHS	6.6±1.26 (4,8) (n=10)		3 (1 7.25) (n=10)		27.6±1.96 (25,30) (n=10)	

Test: Mann Whitney U test; Values: Mean ±SD (min, max) (n) or Median (25% 75%) (n); p<0.05 significant*

Table 6: Table showing follow up scores of patients for all questionnaires at 3 and 6 months

Questionnaire	Range	3 months N (%)	6 months N (%)
GOSE	*1 (Dead)* *2-5* *>5* *Total*	3 (6.82) 15 (34.09) 26 (59.09) 44	0 9 (21.95) 32 (78.04) 41
RHFUQ	<10 >10 Total	29 (70.73) 12 (29.26) 41	33 (80.49) 8 (19.51) 41
QOLIBRI-OS	<18 <18 Total	5(12.2) 36(87.8) 41	4(9.76) 37(90.24) 41

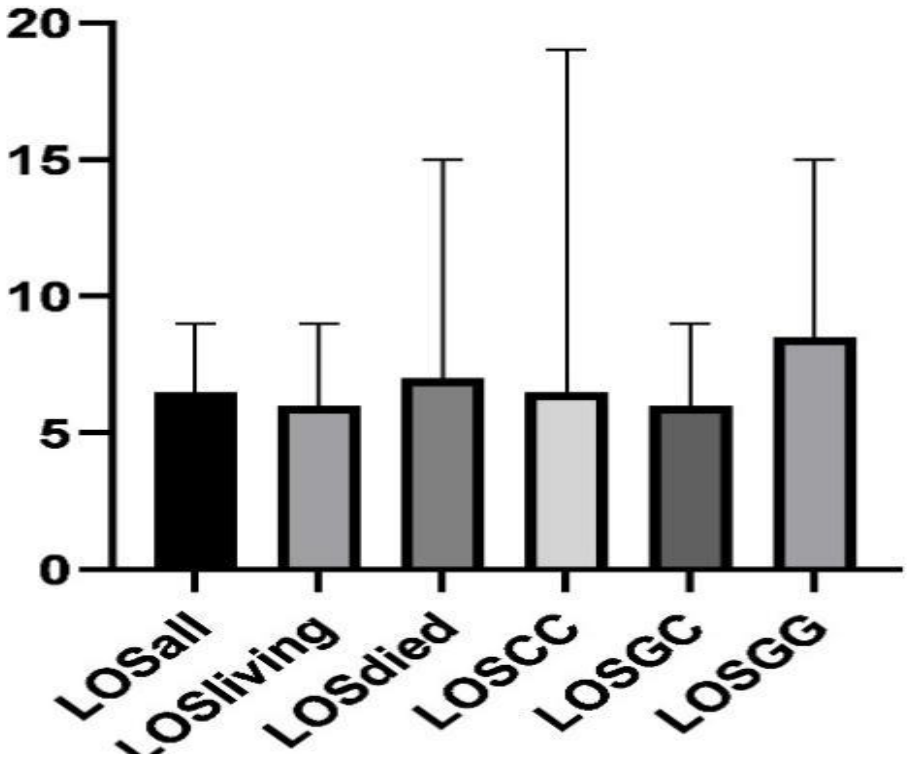

Figure 4: Length of hospital stay among various subgroups of TBI patients (Median).

Table 7: Correlation of length of stay with various outcome scores

		r	p
LOS & GOSE (n=41)	3 months	-0.3645	0.0191*
	6 months	-0.3050	0.05*
LOS & RHFUQ (n=41)	3 months	0.5368	0.0003*
	6 months	0.3366	0.0314*
LOS & QOLIBRI-OS (n=41)	3 months	-0.5253	0.0004*
	6 months	-0.2547	0.108

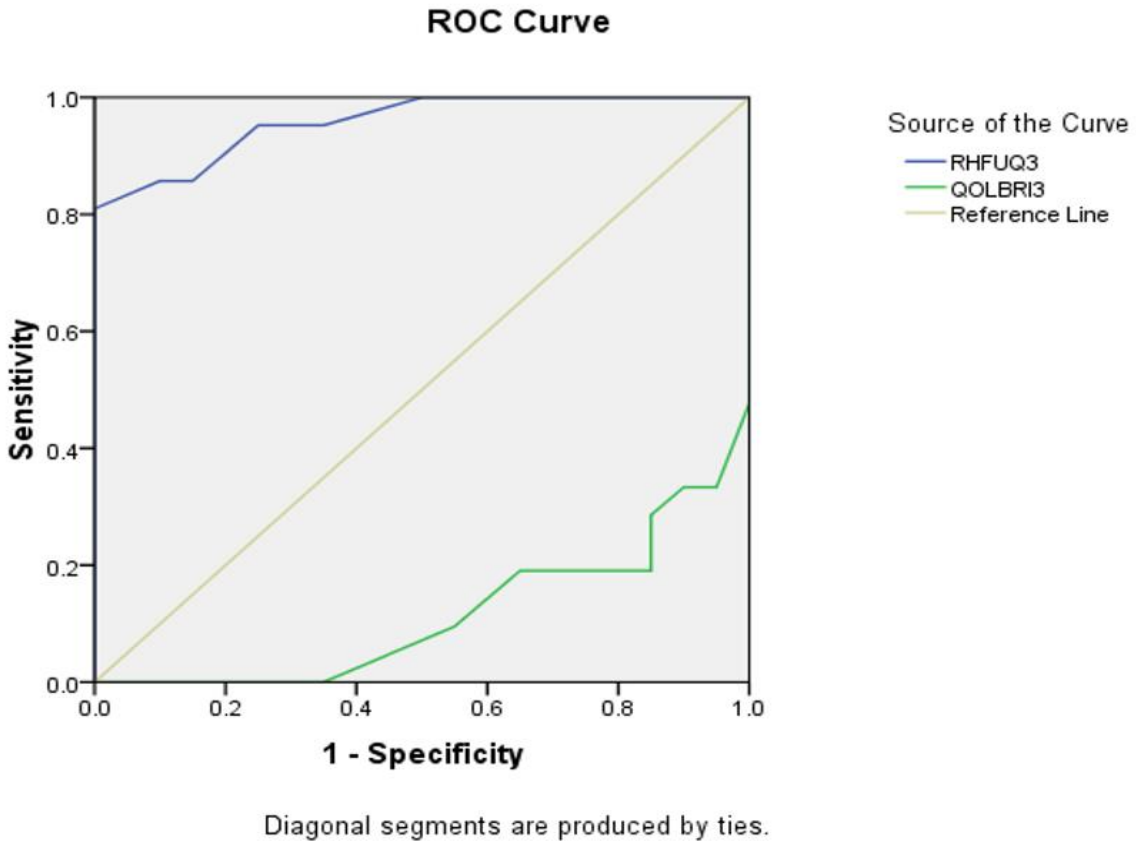

Figure 5: ROC to Assess Functional outcome at 3 months

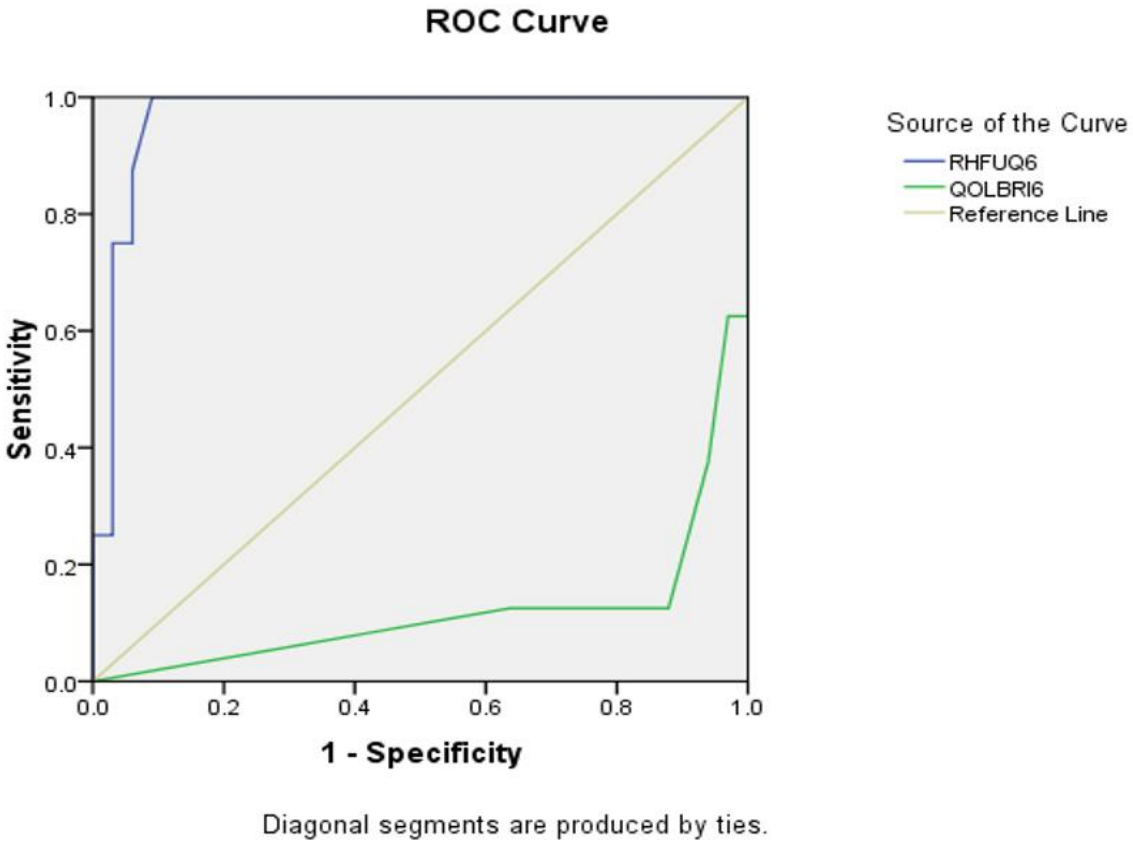

Figure 6: ROC to assess outcome at 6 months

In their study, conducted on 51 Sudanese TBI patients, Mohammed Ali HA et al. discovered comparable findings. Of these patients, 84.31 percent were discharged with a satisfactory outcome. 6.98 percent of patients with good results had Pro/Pro genotypes, 11.62 percent had Arg/Arg genotypes, and 69.76 percent had Pro/Arg genotypes. With 71.42 percent of patients dying and 28.57 percent having a bad prognosis, about 13.72 percent of patients experienced a catastrophic outcome. Most likely, it was impossible to follow up with one patient. Patients with the Arg/Pro and Pro/Pro alleles had a 100% survival rate, according to the study [24]. Eighty percent of the deaths had Arg/Arg alleles, which indicates that arginine-containing variations have a poor prognosis.

4. Conclusions

The current in silico investigation, designed to identify the involvement of p53 gene polymorphisms in TBI, strongly implies that the pathobiology of TBI is influenced by both protein-protein interactions and the harmful consequences of p53 gene mutations. According to the study, there was no connection between the p53 gene polymorphism and functional result following TBI. Patients with the CC genotype (proline/proline) had fewer serious injuries, whereas GG-containing genotypes recovered to the fullest extent possible, which was confirmed by the fact that they spent the longest time in the hospital. When GOSE is the industry standard, RHFUQ was the most precise and sensitive technique to evaluate the functional outcome following TBI.

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