PharmacologyOnLine

Archives • 2021 • vol.2 • 732-752

ORIGINAL ARTICLE

Association of Arg72Pro of p53 Gene Polymorphism with Functional Outcome After Traumatic Brain Injury (TBI) by Functional Independence Measure (FIM)

Sriprajna Mayur[a], Usha Adiga[b]*, Ananthan R[c], Sachidananda Adiga[d]

Nitte (Deemed to be University), [Research Scholar [a], Professor, Department of Biochemistry[b], Neurosurgery[c], Pharmacology[d]], KS Hegde Medical Academy(KSHEMA), Mangalore, Karnataka

Author for Correspondence: Dr. Usha Adiga

Email: ushachidu@yahoo.com

Abstract

Traumatic brain injury (TBI) is one of the leading cause of death and disability in the world. Genetics may play a major role in the functional outcome of TBI which is least explored. Objective of the study was to evaluate the association of p53 gene polymorphism with the functional outcome after TBI in terms of Functional Independence Measures questionnaire (FIM).

Prospective Cohort study was conducted on 58 TBI patients admitted in the Neurosurgery wards of J. K. S. H. C. Hospital. The influence of p53 gene polymorphism on Functional Independence Measures (FIM) scores of TBI patients was studied Genotyping of p53 gene was carried out by PCR-RFLP.Functional outcome after TBI was carried out using Outcome tools like, GOSE and FIM. Study subjects were assessed on admission, discharge, 3 and 6 months of injury. Statistical analysis was carried out using GraphPad Prism 9.

There was no significant difference in the distribution of observed and expected homozygous dominant (GG), heterozygous (CG) and homozygous recessive (CC) alleles among cases and controls [χ^2=0.004 and 0.233 respectively; p>0.05].

The association between p53 polymorphic genotypes and GCS,GOSE, at 3 months and 6 months and FIM on admission, discharge,3 and 6 months was statistically insignificant, chisquare values being 0.432, 2.488, 1.102, 0.803, 0.564, 0.227and 0.140 respectively, p>0.05. FIM was a poor tool for the assessment of outcome at 3 months as well as at 6 months. Area under the curve (AUC) were 0.155 and 0.250 respectively at 3 and 6 months.

There was no significant association between p53 gene polymorphism and functional outcome after TBI. However patients with CC genotype (proline/proline) had less severe injuries, whereas the extent of recovery was maximum in GG containing genotypes, supported by their shortest length of hospital stay.

Keyword: : p53, gene polymorphism, Traumatic Brain Injury, Arginine variant, proline variant, Functional Independence Measures (FIM)

Introduction

Head injury is the most common cause of trauma-related death and severe disability. India has the second largest world population and over a quarter of the world's trauma deaths occur in India. Data from the national crime record bureau report an increase of 63% in accident related deaths over the period 2004-2013 [1]. The total volume of TBI in India is unknown, but estimates suggest that there are more than a million trauma related deaths in India per year, of which 50% are TBI related. The outcome for patients with TBI depends upon the severity of the primary injury and also on the extent of the secondary brain damage. The secondary damage to brain is a neuro inflammatory phenomenon, mediated by the activation of astrocytes and microglia, damage to the blood brain barrier and production of various cytokines. The detailed understanding of these secondary pathophysiologic responses to brain injury offers the prospects of developing new therapeutic modalities. One means of exploring such physiological and pathophysiological pathways is through the use of genetic tools.

Genetic factors, contribute to the causation of brain injury, such as cerebral arterial aneurysms and dementia, as well as influence the outcome after such an injury [2]. Identifying these genetic elements and establishing a causative association of such a gene and traumatic brain injuries is a complicated task as a variety of environmental factors may have a role in the disease pathogenesis as well as outcome determination. The extent of neuronal injury is also influenced by factors like intracranial pressure and cerebral blood volume, cytokine and inflammatory response, tissue oxygen tension, temperature, drug therapies etc. There may be an interaction between these factors and genetics to influence the overall outcome [2]. Hence the identification of genetic element is a difficult task in multi-factorial diseases like TBI.

The p53 tumor suppressor factor is a key regulator of DNA repair, cell cycle progression, apoptosis, and neuronal damage [3]. p53 is induced shortly after TBI, while its inhibition is assumed to offer neuroprotection [4,5]. The polymorphism of p53 gene may play a role in determining the functional outcome after TBI.

The Functional Independence Measure (FIM) is an instrument that was developed as a measure of disability for a variety of populations and is not specific to any diagnosis. The FIM instrument includes measures of independence for self-care, including sphincter control, transfers, locomotion, communication, and social cognition. It is an 18-item, seven-level, ordinal scale intended to be sensitive to changes over the course of a comprehensive inpatient medical rehabilitation program. It uses the level of assistance an individual need to grade functional status from total independence to total assistance. The tool is used to assess a patient's level of disability as well as a change in patient status in response to rehabilitation or medical intervention. This tool may be useful in assessing the functional outcome post-traumatic brain injury.

There have been very few such studies in literature to correlate the association of genes and their polymorphisms, with the outcome after severe traumatic brain injury. Such a study will open new insights into the therapeutic possibilities in TBI. The association of these genes and their polymorphisms with outcome after TBI can be a basis of future therapeutic trials targeting such genes. This will open an entire new era of genetic therapeutic modalities in the management of TBI. Gene polymorphism may be an early marker in predicting the outcome after severe TBI. There are only a few such studies available to the best of our knowledge.

The functional independence measure (FIM) is one of the most widely used measures of function in rehabilitation. It is an 18-item scale used to assess the patient's level of independence in mobility, self-care, and cognition. However, it may lack sensitivity in patients with very low or very high levels of function. Therefore, the FIM may be an inadequate outcome measure for patients at either extreme of TBI recovery. However, FIM has been incorporated in the study to assess the outcome after TBI. The tool was used as one of the functional outcome assessment tools after TBI.

There are only few studies which find the association of the functional outcome and gene polymorphisms in TBI patients to the best of our knowledge.

Objectives

The objectives of the study were to,

1. evaluate the patterns of Arg 72 Pro polymorphism of p53 gene in TBI patients as compared to controls
2. find out whether there is an association of p53 gene polymorphism with the outcome after traumatic brain injury, in terms of extended Glasgow outcome scale(GOSE) and functional independence measure (FIM)

Methodology

The present Cohort study was conducted in the Molecular division of central research laboratory by Department of Biochemistry in collaboration with the Department of Neurosurgery, KSHEMA, Mangalore

Inclusion Criteria:

Traumatic brain injury patients fulfilling following criteria were included

- **i.** patients between the ages of 18-60 years.
- **ii.** patients with mild, moderate or severe injury within 24 hours of injury, after resuscitation and stabilization at admission.

Exclusion Criteria:

TBI patients with following associated conditions were excluded.

- **i.** Anoxic intra-cerebral damage or brain death
- **ii.** Spinal cord injury.
- **iii.** Neurological disorders
- **iv.** Cerebrovascular diseases

Three ml of blood was collected in EDTA tubes after obtaining written informed consent from the patient or their first degree relatives. DNA isolation was done by the salting out method. The quality of the DNA was checked by electrophoresis on 0.8% Agarose gel, containing ethidium bromide (0.5 µg/ml) in TAE buffer. The quantification and purity of DNA was checked by the spectrophotometer (ratio of OD260 / OD280). DNA concentration was calculated using the following formula:

Concentration (µg/ml) of DNA in original solution= Absorbance x 100 x 50 µg/ml.

Amplification and Genotyping of the gene polymorphism: p53 gene was genotyped using PCR-RFLP method. Details of forward and reverse primers, restriction enzymes and reaction conditions are as given in table 1.

Outcome assessment:

Functional outcome after TBI was carried out using Outcome tools like, Glasgow Outcome Scale Extended (GOSE) and Functional Independence Measure (FIM). For all categories of TBI the outcome was assessed at three months and six months post injury.

Statistical analysis:

Statistical analysis was carried out using Graphpad Instat version 3. Hardy Weinberg Equilibrium (HWE) was carried out to evaluate the allelic distribution and $\chi 2$ test was used to assess association between genetic polymorphism and outcome. Kruskal Walli's followed by the post hoc test, Dunn's test was carried out to compare functional outcomes between different allelic variants as well as to compare the scores at admission, discharge, 3 months and 6 months. ROCs were constructed to assess the utility of outcome tools. Correlation of FIM scores with the length of hospital stay in the hospital.

Results

Our study participants were 58 cases and controls with mean age of 38.66± 12.2 (17,62) and 34.3± 12.87 (21,60) respectively. We could not follow up 6 of them as we could not approach them using the contact details shared with us. This brings down number of study subjects to 52 (Figure 1). 3:1 and 4:1

were the ratios for males to females among cases and controls respectively. RTA was the common mode of injury (71.4%), followed by fall (26.19%), assault and other (2.41%). Total number of TBI patients sub grouped as mild, moderate and severely injured according to their GCS score on admission to hospital are depicted in the figure 2.

Genotyping of TP53

Polymerase chain reaction (PCR) resulted in 448 bp fragment. PCR products were subjected to Restriction Fragment Length Polymorphism (RFLP) by digesting with Bstu1 restriction enzyme. Digested products were subjected to gel electrophoresis using 2% agarose gel. Fragments which are undigested (448bp) are CC, fragments with 248 bp are GG and CG are marked by both 448bp and 248bp bands (Figure 3).

We did not find a significant difference between the allelic distribution of genotypes among cases and controls. In cases χ^2= 0.004, p= 0.474, q= 0.529 and in controls χ^2=0.233, p=0.603, q=0.396 (Table 2). Distribution of p53 polymorphic allele did not show a significant gender difference (χ^2 = 2.05, p= 0.151).
The polymorphic genotypes of p53 and GCS score on admission did not show any significant difference (χ^2 0.432, p=0.512). However, GCS was highest among CC genotype. Similarly, extended Glasgow outcome scale (GOSE) scores at 3 and 6 months were not significantly different among all the genotypes with χ^2=2.488 and 1.102 respectively (p>0.05), highest scores were observed among the Proline (CC) containing genotype, suggesting better outcome (Table 3).
FIM scores were recorded on admission, discharge, 3 months and 6 months which even failed to show a significant association with p53 genotypes. (χ^2=0.803, 0.564,0.227 and 0.140 respectively, p>0.05).

Motor and cognition components along with the total scores on admission, discharge, 3 and 6 months follow up were compared among different genotypes. Difference in improvements were analyzed statistically. Except the scores of Cognitive component on admission (p=0.019), no significant difference in scores were noted on comparing the scores of different genotypes. Though not significant, highest scores in every component were scored by CC genotype, followed by CG and GG genotype carriers (Table 4).

Friedman test was applied to analyze the functional outcome in TBI patients (Table 5). The motor, cognition components and total scores on admission, discharge, 3 and 6 months follow-up for each genotype were analyzed. CC genotype carriers showed drastic improvement during discharge compared to admission (47%), but mild improvement was seen at 3 and 6 months of injury as compared to the score at discharge (11.6% and 2.5% respectively). The improvement was statistically significant (p<0.0001*) The similar recovery pattern was seen in patients with heterozygous allele, CG . The percentage improvement in scores were 37.5, 2.9 and 3.5 from admission to discharge , discharge to 3 months and 3 months to 6 months respectively with a significant p= 0.0046. Improvement scores from admission to 6 months post injury were statistically insignificant among GG carriers (p=0.09) (table 5). Cognition component showed significant improvements among CC, CG ang GG carriers (p=0.0014, <0.0001 and 0.0234 respectively). Similarly, total scores were statistically significant among CC, CG and GG carriers with p values <0.0001, <0.0001 and 0.0015 respectively.
There were 6 deaths noted among CG genotype and 5 deaths among GG genotype. Post traumatic incidence of Epilepsy were observed among CG genotype carriers.
There were totally 65.38% of subjects (without considering genotype) scored a total FIM score less than 50% showing maximum dependency on admission. At the time of discharge, the number was brought down to 31.11% which again got reduced to 4.87% during 3 and 6 months' follow-up (Table 6). At six months follow up, there were 95.12% of people displaying minimal dependence.

The utility of the outcome assessment tools in assessing the recovery of patients after TBI was evaluated by ROC.

FIM was a poor tool for the assessment of outcome at 3 months as well as at 6 months. Area under the curve (AUC) were 0.155 and 0.250

respectively at 3 and 6 months. Sensitivities were 38.1% and 15% respectively with a cut off scores of 125 and 121 at 3 and 6 months (Figure 3 and 4).
Length of stay in the hospital (LOS) was the highest for patients with GG median 8.5(5.5, 13.5) as compared to CG 6(4, 9) and CC 6.5 (3.5, 18.3) in days. Extent of improvement was shown to be 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 FIM was nearly double at discharge in GG genotypes ,1.5 times in CG, 1.37 times in CC as compared to admission scores. However, LOS was not statistically significant (p=0.689) in various genotypes.

Correlation of functional scores with the LOS in the hospital is as depicted in table 7.

Significant negative correlation was seen between LOS and total FIM score at discharge, 3 and 6 months. This indicates longer hospital stay among subjects with lower FIM score (Table 7).
The correlation was negative at discharge and 3 months in both CC and GG genotypes. Whereas, it was significant at 6 months as well in patients with CC genotype.

Discussion

The p53 has different genotypes expressions, homozygotic Arg72Arg (wild type), heterozygous Arg72Pro, and homozygous Pro72Pro (mutant varieties). Based on the presence of proline or arginine, they can be homozygous dominant(GG), heterozygous(CG) and homozygous recessive(CC). G containing alleles code for arginine and C containing alleles code for proline.

In our study, predominance of 'C' alleles were observed in controls (Table 2). Whereas in cases 'G' allele was predominant. However, there was no significant difference between the observed and the expected allele frequencies as per HWE results (p>0.05).

The detection of the genotypes variants is due to the difference in their electrophoretic mobility [6]. The single nucleotide polymorphism of p53 has been implicated to be play a major role in the functional outcome of patients with TBI [7]. Marked functional differences have been reported between the genotypes forms, Arg/Arg and Arg/Pro of the p53-protein [8-11]. The Arg72Arg form is found to have a key role in inducing apoptosis. Arg72Pro form has a main role in activating p53 dependent DNA repair by arresting cell cycle at G1 phase [12-15].This fact justifies the protective role of proline containing allele in TBI.

The frequency of the expression of SNP of p53, exhibit variability based on ethnicity.Highest expression of proline was seen among South Africans(70%) and 23% among Western Europeans. Pro72Pro(CC) allele may be of protective value against sunlight-induced diseases as suggested by the latitude gradient from Europe to Africa [16], however, contradictory studies are available on risk of skin malignancy [17,18].

The reduced mortality in Pro/Pro homozygotes vs Arg/Pro heterozygotes and Arg/Arg homozygotes, which could result from a generally increased robustness caused by decreased pro-apoptotic activity and increased cell cycling arresting abilities of the Pro72 versus the Arg72 version of p53, thereby protecting a person experiencing any critical illness [9,13,19].

Severity of injury as assessed by GCS, was highest in patients with CC (1.2-1.3 times) compared to other genotypes (through insignificant). This suggests an insignificant association between the severity of injury and CC genotype of p53 (table 3). GOSE scores were also the highest in CC genotype at 3 months and 6 months suggestive of a better recovery in proline containing genotypes. Patients with CG and GG showed lower moderate disability at 3 months and upper-moderate disability at 6 months, whereas CC genotype showed upper moderate disability at 3 months and lower good recovery at 6 months.

Extended Glasgow outcome scale (GOSE) is a more sensitive and preferred tool compared to Glasgow outcome scale, which is taken as gold standard tool to assess the outcome in our study.

Recovery after TBI is heterogeneous and depends on the age of the patient, and the nature, location, and extent of the injury [20,21]. The known

predictors account for only a limited percentage of the variation in outcomes. We can only emphasize that genetic factors influence the outcome as in addition to other factors.

The FIM questionnaire assesses motor as well as cognition components separately the scores being 91 and 35 respectively with a total score of 126. When motor component of FIM was analyzed in the three genotypes a highly significant increment was noted in the scores at admission, discharge 3 months and 6 months for CC and CG (p value being <0.0001*0.0046*respectively) and insignificant increment was noted for GG p=0.09 respectively) (table5).

At admission higher FIM motor score was seen in CC genotypes and lowest in GG genotypes. But at the end of 6 months, CC, CG and GG genotypes had similar score suggesting a better recovery in patients with all groups by 6 months irrespective of genotype (table 4).

Scores difference between discharge and admission was significant in all genotypes (p<0.0001). The difference was insignificant for 3 and 6 months. On comparing overall difference in FIM motor scores of admission compared to discharge, 3 months and 6 months. A significance (p= 0.0046) was obtained (table 5). Difference score almost doubled at 6 months compared to discharge, suggesting overall better wellbeing at 6 months.

FIM total scores were insignificantly different among all the three genotypes when compared at admission, discharge, 3 months and 6 months (table 5). Admission total score was the highest in CC, 1.2 times and 1.7 times compared to CG and GG. This implies a less susceptibility of CC alleles for injury. GG genotypes were at higher risk of severe injury. However, recovery rate was better in GG genotypes at 3 months and 6 months as shown by the high score.

Utility of the FIM questionnaire at 3 and 6 months to assess the recovery of TBI patients, taking GOSE as gold standard was analyzed by ROC (fig 3&4). Curves lied below the diagonal line, suggesting a poor accuracy. Low area under the curve, sensitivity and specificity suggest that FIM may not be a suitable tool for assessing functional outcome of TBI.

Patients with CC genotype required moderate assistance at admission as per FIM scale interpretation. The conditions remained the same (even-though scores improved) at discharge and 3 months. Clinical improvement was observed at 6 months as they moved to minimal assistance zone.

Patients with CC genotype required moderate assistance at admission as per FIM scale interpretation. The conditions remained the same (even-though scores improved) at discharge and 3 months. Clinical improvement was observed at 6 months as they moved to minimal assistance zone.

CG and GG genotypes needed maximum assistance at admission. At discharge they moved to moderate assistance category and to minimum assistance category at 3 months. This fact implies that patients irrespective of genotype, showed maximum improvements at 3 and 6 months follow up. Similar pattern of recovery was observed in cognitive scores and total FIM scores. A significant negative correlations observed between FIM total scores and LOS suggest a prolonged length of hospital stay in patients with slower recovery. this pattern was more marked in patients with CC and GG genotypes (Table 7).

The p53 tumor suppressor factor is a key regulator of DNA repair, cell cycle progression, apoptosis, and neuronal damage. p53 is induced shortly after TBI, while its inhibition is assumed to offer neuro-protection [3-5,24]. p53 tumor suppressor gene because of its pro-apoptosis property and has been reported to have human longevity, cancer risk and survival [25,30]. It has been acknowledged that p53 contributes to neuronal cell apoptosis and autophagy, as well as trans-activating genes that play a role in neuronal cell repair and regeneration, supporting warranting investigation into of the relationship between p53 and TBI outcomes [28,31,32]. Exploratory animal studies have also identified changes in expression of p53 following closed head injury, ischemic brain injury, lateral fluid

percussion mediated injury and p53 deficient mice [7, 33-36]. Specifically, these animal models have found an upregulation of p53 in the nucleus of injured cells following TBI [35].

All these evidences support the role of p53 gene in determining the functional outcome after TBI.

Conclusion

It could be concluded from the study that there was no significant association between p53 gene polymorphism and functional outcome after TBI in terms of FIM scores. However, patients with CC genotype (proline) had less severe injuries, whereas the extent of recovery was maximum in GG containing genotypes. FIM was not a sensitive and specific tool to assess the functional outcome after TBI, as compared to GOSE as the gold standard.

References

1. Deaths A. National Crime Records Bureau. Ministry of Home Affairs.
2. Wilson M, Montgomery H. Impact of genetic factors on outcome from brain injury. British journal of anaesthesia. 2007 Jul 1;99(1):43-8.
3. Culmsee C, Mattson MP. p53 in neuronal apoptosis. Biochemical and biophysical research communications. 2005;331(3):761-77.
4. Napieralski JA, Raghupathi R, McIntosh TK. The tumor-suppressor gene, p53, is induced in injured brain regions following experimental traumatic brain injury. Molecular brain research. 1999;71(1):78-86
5. Xue L, Yang SY. The protective effect of p53 antisense oligonucleotide against neuron apoptosis secondary to traumatic brain injury. Zhonghua wai ke za zhi [Chinese Journal of Surgery]. 2004;42(4):236-9
6. Harris N, Brill E, Shohat O, Prokocimer M, Wolf D, Arai N, Rotter V. Molecular basis for heterogeneity of the human p53 protein. Molecular and cellular biology. 1986;6(12):4650-6.
7. Martínez-Lucas P, Moreno-Cuesta J, García-Olmo DC, Sánchez-Sánchez F, Escribano-Martínez J, del Pozo AC, Lizán-García M, García-Olmo D. Relationship between the Arg72Pro polymorphism of p53 and outcome for patients with traumatic brain injury. Intensive care medicine. 2005;31(9):1168-73.
8. Marin MC, Jost CA, Brooks LA, Irwin MS, O'Nions J, Tidy JA, James N, McGregor JM, Harwood CA, Yulug IG, Vousden KH. A common polymorphism acts as an intragenic modifier of mutant p53 behaviour. Nature genetics. 2000;25(1):47-54.
9. Dumont P, Leu JJ, Della Pietra AC, George DL, Murphy M. The codon 72 polymorphic variants of p53 have markedly different apoptotic potential. Nature genetics. 2003;33(3):357-65.
10. Moreau F, Matlashewski G. Molecular analysis of different allelic variants of wild-type human p53. Biochemistry and Cell Biology. 1992;70(10-11):1014-9.
11. Sullivan A, Syed N, Gasco M, Bergamaschi D, Trigiante G, Attard M, Hiller L, Farrell PJ, Smith P, Lu X, Crook T. Polymorphism in wild-type p53 modulates response to chemotherapy in vitro and in vivo. Oncogene. 2004;23(19):3328-37.
12. Siddique M, Sabapathy K. Trp53-dependent DNA-repair is affected by the codon 72 polymorphism. Oncogene. 2006;25(25):3489-500.
13. Bergamaschi D, Samuels Y, Sullivan A, Zvelebil M, Breyssens H, Bisso A, Del Sal G, Syed N, Smith P, Gasco M, Crook T. iASPP preferentially binds p53 proline-rich region and modulates apoptotic function of codon 72–polymorphic p53. Nature genetics. 2006 ;38(10):1133-41.
14. Storey A, Thomas M, Kalita A, Harwood C, Gardiol D, Mantovani F, Breuer J, Leigh IM, Matlashewski G, Banks L. Role of a p53 polymorphism in the development of human papilloma-virus-associated cancer. Nature. 1998;393(6682):229-34.
15. Thomas M, Kalita AN, Labrecque S, Pim D, Banks L, Matlashewski G. Two polymorphic variants of wild-type p53 differ biochemically

and biologically. Molecular and cellular biology. 1999;19(2):1092-100
16. Beckman G, Birgander R, Själander A, Saha N, Holmberg PÅ, Kivelä A, Beckman L. Is p53 polymorphism maintained by natural selection? Human heredity. 1994;44(5):266-70.
17. Bastiaens MT, Struyk L, Tjong-A-Hung SP, Gruis N, ter Huurne J, Westendorp RG, Vermeer BJ, Bavinck JN, ter Schegget J. Cutaneous squamous cell carcinoma and p53 codon 72 polymorphism: a need for screening?. Molecular Carcinogenesis: Published in cooperation with the University of Texas MD Anderson Cancer Center. 2001;30(1):56-61.
18. Han J, Cox DG, Colditz GA, Hunter DJ. The p53 codon 72 polymorphism, sunburns, and risk of skin cancer in US Caucasian women. Molecular Carcinogenesis: Published in cooperation with the University of Texas MD Anderson Cancer Center. 2006 ;45(9):694-700.
19. Pim D, Banks L. p53 polymorphic variants at codon 72 exert different effects on cell cycle progression. International journal of cancer. 2004;108(2):196-9.
20. Polinder S, Haagsma JA, van Klaveren D, Steyerberg EW, Van Beeck EF. Health-related quality of life after TBI: a systematic review of study design, instruments, measurement properties, and outcome. Population health metrics. 2015;13(1):1-2.
21. Kim YJ. A systematic review of factors contributing to outcomes in patients with traumatic brain injury. Journal of clinical nursing. 2011;20(11-12):1518-32.
22. JA CH, Jordán J, DC GC. Evaluation of the p53 Arg72Pro polymorphism as a prognostic factor in severe head injury and the inclusion of this indicator in a predictive model. Revista espanola de anestesiologia y reanimacion. 2009;56(9):529-35.
23. Mohammed Ali HA, Sawsan Aldeaf AH, Ehassan SH, Gassoum A, Abdrabo AA. Role of p53 Gene Arg72Pro and serum electrolytes in outcome of traumatic brain injury among Sudanese patients. International Journal of Recent Scientific Research. 2016;7(5):11021-27.
24. Dumont P, Leu JJ, Della Pietra AC, George DL, Murphy M. The codon 72 polymorphic variants of p53 have markedly different apoptotic potential. Nature genetics. 2003;33(3):357-65
25. TP53 gene. 2018; Available from: https://ghr.nlm.nih.gov/gene/TP53#location.
26. Beckerman R, Prives C. Transcriptional regulation by p53. Cold Spring Harbor perspectives in biology. 2010;2(8):a000935.
27. Toledo F, Wahl GM. Regulating the p53 pathway: in vitro hypotheses, in vivo veritas. Nature Reviews Cancer. 2006;6(12):909-23.
28. Wan C, Ma X, Shi S, Zhao J, Nie X, Han J, Xiao J, Wang X, Jiang S, Jiang J. Pivotal roles of p53 transcription-dependent and-independent pathways in manganese-induced mitochondrial dysfunction and neuronal apoptosis. Toxicology and applied pharmacology. 2014;281(3):294-302.
29. Zhang J, Cui Z, Feng G, Bao G, Xu G, Sun Y, Wang L, Chen J, Jin H, Liu J, Yang L. RBM5 and p53 expression after rat spinal cord injury: implications for neuronal apoptosis. The international journal of biochemistry & cell biology. 2015;60:43-52.
30. Ørsted DD, Bojesen SE, Tybjærg-Hansen A, Nordestgaard BG. Tumor suppressor p53 Arg72Pro polymorphism and longevity, cancer survival, and risk of cancer in the general population. The Journal of experimental medicine. 2007;204(6):1295-301.
31. Wan C, Jiang J, Mao H, Cao J, Wu X, Cui G. Involvement of upregulated p53-induced death domain protein (PIDD) in neuronal apoptosis after rat traumatic brain injury. Journal of Molecular Neuroscience. 2013;51(3):695-702.
32. Napieralski JA, Raghupathi R, McIntosh TK. The tumor-suppressor gene, p53, is induced in injured brain regions following experimental traumatic brain injury. Molecular brain research. 1999;71(1):78-86.
33. Lu J, Moochhala S, Kaur C, Ling EA. Changes in apoptosis-related protein (p53, Bax, Bcl-2 and Fos) expression with DNA

fragmentation in the central nervous system in rats after closed head injury. Neuroscience letters. 2000;290(2):89-92.
34. Chopp M, Li Y, Jiang N. Increase in apoptosis and concomitant reduction of ischemic lesion volume and evidence for synaptogenesis after transient focal cerebral ischemia in rat treated with staurosporine. Brain research. 1999;828(1-2):197-201.
35. Kaya SS, Mahmood A, Li Y, Yavuz E, Göksel M, Chopp M. Apoptosis and expression of p53 response proteins and cyclin D1 after cortical impact in rat brain. Brain research. 1999;818(1):23-33.
36. Morrison RS, Wenzel HJ, Kinoshita Y, Robbins CA, Donehower LA, Schwartzkroin PA. Loss of the p53 tumor suppressor gene protects neurons from kainate-induced cell death. Journal of Neuroscience. 1996;16(4):1337-45.
37. Chen AY, Colantonio A. Defining neurotrauma in administrative data using the International Classification of Diseases Tenth Revision. Emerging themes in epidemiology. 2011;8(1):1-3.

Table 1: Reaction conditions for PCR-RFLP technique 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' CCTGAAAACAAC GTTCTGGTAA 3' 5' GCATTGAAGCTC CATGGAAG 3'	94°C, 5" 94°C,30', 55°C,30', 72°C,30' 72°C, 7"	448bp	BstUI, 37°C	248 bp

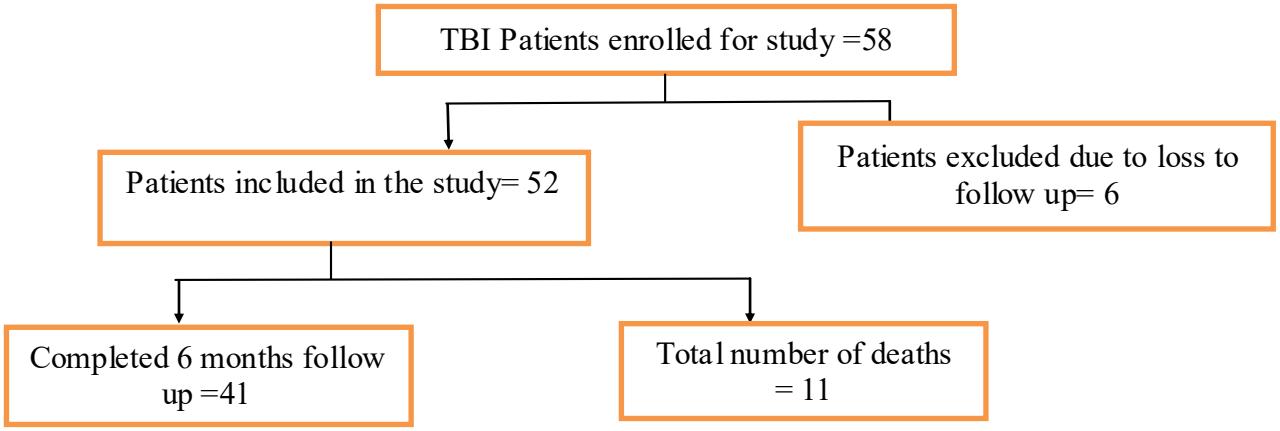

Figure 1: Flowchart depicting patients included in the study.

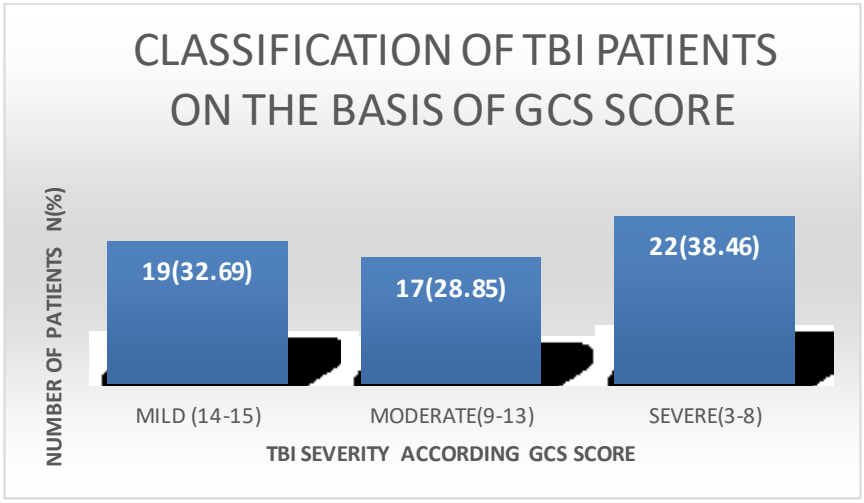

Figure 2: Bar diagram showing classification of TBI patients under severity subgroups on the basis of GCS score on admission to hospital.

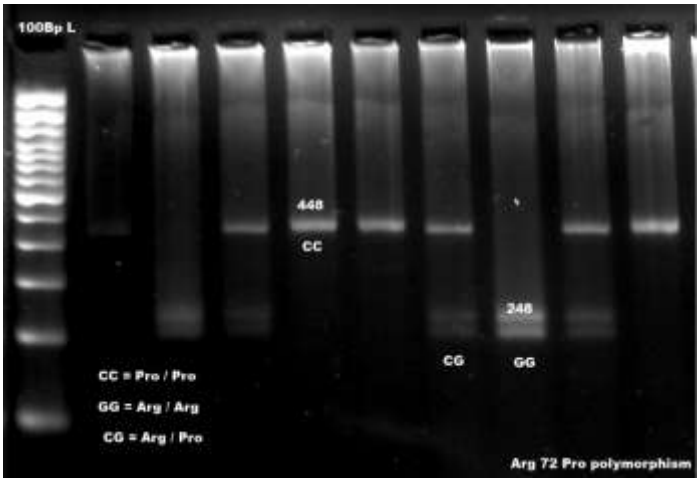

Figure 3: Agarose gel electrophoresis of RFLP digested p53 PCR with BstU1 restriction endonuclease

Table 2: Showing the Allelic Distribution of p53 Arg72 Pro in 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 3: Comparison of scores of various scales in TBI with respect to p53 genotype.

p53 genotype	CC Mean ±SD (n)	CG Mean ±SD (n)	GG Mean ±SD (n)	p value
GCS on Admission (N= 58)	15 (8.25- 15) (n=12)	12 (6.5- 15) (n=29)	9 (5 - 11) (n=16)	0.127
GOSE- 3 months follow up (N=41)	7 (4.5 – 8) (n=12)	6 (4 – 7) (n=19)	4 (3 – 7) (n=11)	0.06
GOSE- 6 months follow up (N=41)	8 (7 - 8) (n=12)	7 (4 - 8) (n=18)	6.5 (6 - 8) (n=11)	0.26

Table 4: Comparison of FIM scores among different genotypes

FIM	TIME OF SCORING	CC	CG	GG	*P* value
Motor score	Admission	49.5 (15 – 71.8) (n=12)	13 (13 – 68.5) (n=25)	13 (13 – 31) (n=15)	0.084

	Discharge	81 (36.8 – 91) (n=12)	70 (28.3 – 91) (n=20)	59.5 (19- 85.5) (n=12)	0.113
	3 months	91 (59 - 91) (n=12)	91 (69 - 91) (n=19)	91 (81 – 91) (n=10)	0.404
	6 months	91 (67 – 91) (n=12)	91 (91- 91) (n=19)	91 (91- 91) (n=10)	0.68
Cognition score	Admission	31.5 (7.5, 35) (n=12)	5 (5,32.5) (n=25)	5 (5, 10) (n=15)	0.019*
	Discharge	35 (28.3, 35) (n=12)	29 (9.25, 39) (n=20)	27.5(6.25,34.5) (n=12)	0.197
	3 months	35 (35, 35) (n=12)	35 (33, 35) (n=19)	35 (33, 35) (n=10)	0.82
	6 months	35 (35, 35) (n=12)	35 (35, 35) (n=19)	35 (35, 35) (n=10)	0.8
Total score	Admission	81 (22.7,107) (n=12)	18 (18,101) (n=25)	18 (18,41) (n=15)	0.067
	Discharge	113 (71.8,126) (n=12)	97.5 (36.8, 126) (n=20)	87 (25.3, 117) (n=12)	0.30
	3 months	126 (88,126) (n=12)	126 (102,126) (n=19)	126 (108,126) (n=10)	0.90
	6 months	126 (98,126) (n=12)	126 (126,126) (n=19)	126 (126,126) (n=10)	0.38

Data represented as Median IQR(25%, 75%). *Reduced number of participants from admission to discharge was due to deaths, at 3 months follow up were due to deaths and loss to follow up.
Test : Kruskal wallis.

Table 5: Comparison Improvements in FIM score with every follow up with respect to p53 Genotypes Data represented as Median IQR(25%, 75%) . *Reduced number of participants from admission to discharge was due to deaths, at 3 months follow up were due to deaths and loss to follow up. Test :Friedman test.

FIM-Component	p53 genotype	Admission	Discharge	3 month	6 month	P value
MOTOR	CC	49.5 (15 –71.8) (n=12)	81 (36.8 – 91) (n=12)	91 (59 - 91) (n=12)	91 (67 -91) (n=12)	<0.0001*
	CG	13 (13 – 68.5) (n=25)	70 (28.3 – 91) (n=20)	91 (69 - 91) (n=19)	91 (91- 91) (n=19)	0.0046*

	GG	13 (13 – 31) (n=15)	59.5 (19-85.5) (n=12)	91 (81 – 91) (n=10)	91 (91- 91) (n=10)	0.09
COGNITION	CC	31.5 (7.5, 35) (n=12)	35 (28.3, 35) (n=12)	35 (35, 35) (n=12)	35 (35, 35) (n=12)	0.0014 *
	CG	5 (5,32.5) (n=25)	29 (9.25, 39) (n=20)	35 (33, 35) (n=19)	35 (35, 35) (n=19)	<0.0001 *
	GG	5 (5, 10) (n=15)	27.5(6.25,34.5) (n=12)	35 (33, 35) (n=10)	35 (35, 35) (n=10)	0.0234*
TOTAL	CC	81 (22.7,107) (n=12)	113 (71.8,126) (n=12)	126 (88,126) (n=12)	126 (98,126) (n=12)	<0.0001 *
	CG	18 (18,101) (n=25)	97.5 (36.8, 126) (n=20)	126 (102,126) (n=19)	126 (126,126) (n=19)	<0.0001 *
	GG	18 (18,41) (n=15)	87 (25.3, 117) (n=12)	126 (108,126) (n=10)	126 (126,126) (n=10)	0.0015 *

Table 6: Number of TBI patients distributed under FIM score categories of dependence.

FIM TOTAL SCORES	MAXIMUM DEPENDENCE (<25-50%) N (%)	MODERATE DEPENDENCE (51-75%) N (%)	MINIMAL DEPENDENCE (76-100%) N (%)	TOTAL SUBJECTS
ON ADMISSION	34 (65.38)	4(7.69)	14(26.92)	52
ON DISCHARGE	14(31.11)	6(13.63)	24(54.54)	44
AT 3 MONTHS	2(4.87)	4(9.75)	35(85.36)	41
AT 6 MONTHS	2(4.87)	0	39(95.12)	41

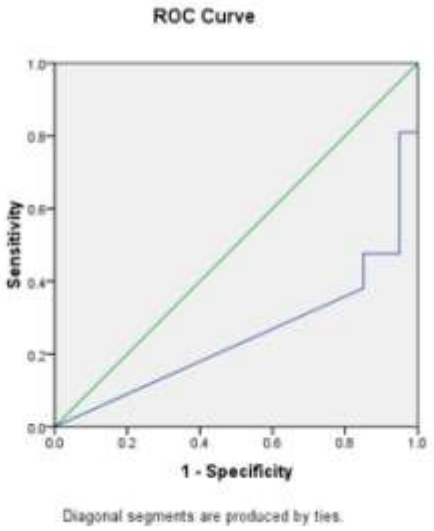

Fig 3: ROC to Assess Functional outcome at 3 months

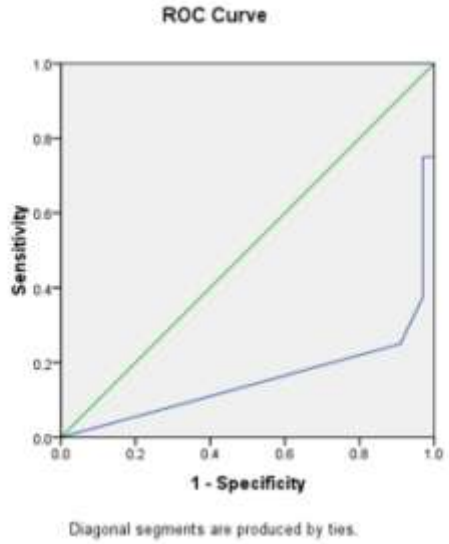

Fig 4: ROC to assess outcome at 6 months

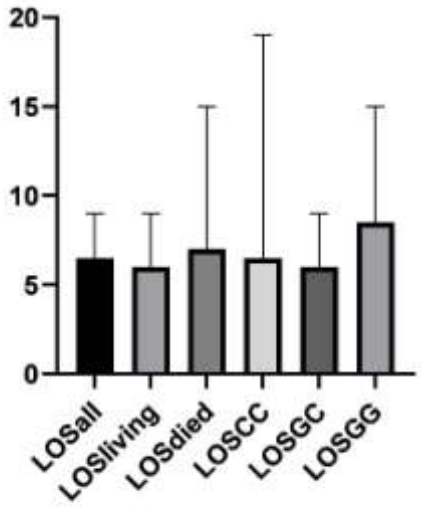

Figure 5: Median length of stay in hospital among various subgroups of TBI.

Table 7: Correlation of length of stay with various components of FIM scores

LOS in days	FIM Discharge		FIM 3 months		FIM 6 months	
	r	*p*	r	*p*	r	*p*
ALL patients	-0.6309	<0.0001*	-0.5718	<0.0001*	-0.4945	0.0010*
CC	-0.8114	0.002*	-0.8311	0.0004*	-0.8227	0.0008*
CG	-0.4227	0.0714	-0.3661	0.123	-0.2951	0.22
GG	-0.7378	0.018*	-0.7554	0.018*	-0.2910	0.5