EVALUATION OF SERUM S100B AS A BIOMARKER OF HEAD INJURY IN PATIENTS ADMITTED INTO A NEUROLOGICAL WARD AT PARIRENYATWA HOSPITAL, HARARE, ZIMBABWE

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE MASTERS DEGREE IN CLINICAL BIOCHEMISTRY

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ABSTRACT

Background: - CT scanning is the gold standard for head injury diagnosis. However, the high costs of imaging in Zimbabwe makes it difficult to manage patients with head injuries as most admitted in public hospital are unable to pay for the services. Use of cheaper serum biomarkers may improve patient management. Serum S100B has been introduced as a clinical tool for diagnosis of traumatic brain injury in some emergency departments in European healthcare facilities and has been reported as reducing the frequency of unnecessary CT scans.

Aim: - To validate the clinical utility of serum S100B in patients with head injury.

Materials and methods: - A cross sectional study in which 50 patients with suspected head injuries, 20 apparently healthy health workers and 20 non neurological patients in medical wards were enrolled at Parirenyatwa hospital. Blood samples were withdrawn from head injury suspects within 24 hours of admission and leftover serum samples for the non neurological patients were collected from Parirenyatwa Hospital laboratory. Serum S100B levels were measured and results were correlated with CT scan findings, Glasgow coma scale and Glasgow outcome scale obtained from audit reports. Results from head injury suspects were also compared with those of apparently healthy participants and those from patients with non neurological conditions.

Results: - Serum S100B levels were significantly higher in head injury patients [median 173.25 (IQR 50- 428.1)] compared to apparently healthy participants [median 8.3 (IQR 5.25- 9.8)] and non neurological participants [median 12.75 (IQR 12.75- 18.2)] p <0.001. Of the 50 head injury patients only 29 had a CT scan done of which 25 were positive.
Median S100B levels were significantly higher in patients with positive CT scan [median 174.0 (IQR 100.4- 298.0)] compared to those with negative CT scan [median 10.5 (IQR 7.0-14.0)] p=0.003. Sensitivity and Specificity of S100B for head injury were determined at 3 cut off points and at 40pg/ml was 88% and 100% respectively, at 50pg/ml was 84% and 100%, and at 100pg/ml was 80% and100%. There was a moderately strong negative correlation between GCS and S100B levels (r= -0.39).

**Conclusion:** - The current study demonstrated that measurement of serum S100B levels can be adopted as a biomarker of head injury to reduce unnecessary CT scanning.
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LIST OF ABBREVIATIONS

AHP: Apparently Healthy Participant

ANOVA: Analysis of Variance

CK-BB: Creatine Kinase Brain Isoenzyme

CSF: Cerebral Spinal Fluid

CT: Computed Tomography

ELISA: Enzyme-Linked Immunoabsorbant Assay

GCS: Glasgow Coma Scale

GFAP: Glial Fibrilary Acidic Protein

GOS: Glasgow Outcome Score

HIP: Head Injury Participant

ICP: Intra Cranial Pressure

ISS: Injury Severity Score

JREC: Joint Research Ethics Committee

MBP: Myelin Basic Protein

MRCZ: Medical Research Council of Zimbabwe

MVA: Motor Vehicle Accident
NNCP: Non-Neurological Participant
NSE: Neuron-Specific Enolase
Rpm: Revolutions Per Minute
SD: Standard Deviation
TBI: Traumatic Brain Injury
TMB: Tetramethylbenzidine
WHO: World Health Organisation
IQR: Interquartile Range
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CHAPTER 1

1.0 BACKGROUND AND LITERATURE REVIEW

Traumatic brain injury (TBI) constitutes a major health and socioeconomic problem throughout the world (1). In developed countries TBI is the leading cause of mortality and disability among young people. Globally the incidence of TBI is rising as a result of the increased use of motor vehicles in low and middle income countries (2). The World Health Organisation (WHO) has projected that by 2020 road traffic accidents will be the third largest cause of the global burden of disease and injury. Interpersonal violence also contributes 7-10% cases of closed head injuries (3).

1.1 Classification of TBI

Traumatic brain injury can occur without other associated injuries or it can be associated with injuries not related to the head such as limb fractures, thoracic or abdominal injuries. When TBI is associated with extra cranial injuries there is increased risk of secondary brain damage due to hypoxia, hypotension, pyrexia or coagulopathy (4). The recording of the severity of extra cranial injuries form part of the TBI classification panel known as the injury severity score (ISS) (5). Over the years TBI has been commonly classified by mechanism of injury, by clinical severity (Glasgow coma scale.GCS) and by assessment of structural damage (6).

1.1.1 Mechanism of injury

Classification of TBI according to mechanism of injury is divided into closed and penetrating head injury. A closed injury occurs when the head injury does not expose the brain.

A penetrating head injury occurs when an object pierces the skull and breaches dura matter exposing the brain. The primary causes of closed head injuries are motor vehicle accidents,
falls, acts of violence and sports injuries. Penetrating head injuries are mainly caused by
gunshots and are usually fatal (7).

1.1.2 Clinical severity: Level of consciousness (Glasgow coma scale)

The Glasgow coma scale (GCS) classification system for the severity of TBI consists of the
score that ranges from 3-15 for three components: eye, motor and verbal function (8). For
assessment of severity the three components are reported separately and GCS consists of the
sum score. Injuries are classified based on the total score of the three components as severe
(GCS 3-8), moderate (GCS 9-13) or mild (GCS 14-15). For the eyes the grading is from 1-4
where 1 is given when there is no eye response to any stimulus, 2- when eyes open in
response to painful stimuli, 3- when eyes open in response to speech and 4- when the eye
response is spontaneous. The motor component is graded from 1-6 where grade 1- there is no
motor response, 2- there is a response of extension to painful stimuli, 3 there is abnormal
flexion to painful stimuli, 4- there is flexion or withdrawal to painful stimuli, 5- there is
response to localised stimuli and 6- patient obeys commands. The verbal component is
graded from 1-5 where 1- there is no verbal response, 2- when patient makes
incomprehensible sounds, 3- when patient uses inappropriate words or incoherent language,
4- the patient is showing signs of disorientation and confusion but can speak coherently, 5-
the patient is oriented and converses normally (9).

1.1.3 Neuroimaging

The level of consciousness which is used interchangeably as the GCS is affected by
confounders such as medical sedation, paralysis or intoxication.

Some of the patients presenting at the emergency are heavily intoxicated with alcohol. Since
alcohol impairs the response to eye, motor and verbal function used in GCS the resultant
initial GCS are not usually accurate for such patients (10). The GCS just categorise patients as severe, moderate and mild without differentiating patients within the same group. Failure by the GCS to provide information on the pathologic mechanisms responsible for the neurological defects limits its use in providing targeted interventions. Assessment of structural damage is not affected by the confounders that affect the GCS and also specify the damage. Marshall Computed Tomography (CT) scan classification which assesses structural damage focuses on the presence or absence of a mass lesion. This classification also differentiates diffuse injuries by signs of increased intra cranial pressure (ICP) (11).

Structural damage is classified into six categories. The normal CT scan is categorised as diffuse injury 1 where there is no visible pathology. In diffuse injury 11 cisterns are present, midline shift 0-5 mm and/ or lesion densities or no mass lesion >25cm³. May include bone fragments or foreign bodies. In diffuse injury 111 there is swelling of the brain, cisterns compressed or absent with midline shift 0-5mm or no mass lesion >25cm³. In diffuse injury 1V there is a shift, midline shift >5mm, no mass lesion >25cm³. Any lesion surgically evacuated is categorised as evacuated mass lesion. High or mixed-density lesion >25cm³ not surgically evacuated is categorised as non-evacuated mass lesion (11). The Marshall classification has limitations. The classification broadly differentiates between diffuse and mass lesions and also does not specify the type of mass lesion for example it does not specify whether the mass lesion is epidural or subdural (12). Thus this classification can fail to differentiate patients with diffuse axonal injury or signs of raised ICP in addition to a mass lesion.

Furthermore CT can only capture momentary dynamic processes but lesions that occur at microscopic level such as diffuse axonal injury and ischemic changes cannot be visualised (13).
1.2 Glasgow outcome scale

The Glasgow outcome scale (GOS) is a 5 point score given to head injury patients at some point of recovery. The GOS is graded as 1- death, 2- vegetative state, 3- severe disability, 4- moderate disability and 5- good recovery (14). An early prediction of outcome is of paramount importance as this allows for informed counselling of relatives and also helps the physician to decide on patient management (15). It has always been thought that patients with GCS of 3 do not survive but in a study done by Chamoun R B et al (2009) some patients survived when aggressive treatment was done indicating that a low GCS is not always associated with poor outcome (16).

1.3 Biomarkers of head injuries.

Glasgow coma scale, pupil reactivity and CT scans are some of the primary clinical indicators currently being used for diagnosis of brain injury. Although the clinical indicators have proved to be useful they have limited utility in predicting adverse secondary events and detecting subtle damage (17). Biomarkers reflecting a biological response to injury or a disease process have proven useful for the diagnosis of many pathological conditions (18). Potential biomarkers that have been proposed for head injury are proteins synthesized in astroglial cells or neurons of the brain.

The proteins include CK-BB (the Creatine kinase isoenzyme predominant in the brain), glial fibrillary acidic protein (GFAP), myelin basic protein (MBP), neuron-specific enolase (NSE) and S100B (19). Literature reports some biomarkers such as S100B protein having high sensitivity for brain injury, possibly even higher than CT scanning (20).
1.3.1 Creatine kinase-BB

Creatine kinase-BB predominantly occurs in the central nervous system (CNS) although under physiological conditions minute quantities also occur in the gastrointestinal system, uterus and vascular wall (21). Any anatomical injury to brain tissue causes release of CK-BB from the astrocytes of the brain (22). Increased activity of enzyme is not specific for head injuries as increased activity also occurs in adenocarcinomas of the prostate, breast, ovary, colon, and other adenocarcinomas of the gastrointestinal tract and small cell anaplastic carcinoma of the lungs (19). Serum activity of CK-BB increases during the first hours after trauma and drop quickly unless there is continued enzyme release (23). A pilot study by ME Carr Junior (2009) reported that serum CK BB had a low sensitivity (11%) for the detection of head injury. The findings were also not correlated with the extent of injury (24). In cerebral spinal fluid (CSF) samples correlation between outcome and lesion size with CK-BB activity has been reported but the same correlation has not been found in serum. The lack of diagnostic accuracy in serum samples is thought to be due to either low transfer rates of CK-BB from CSF to serum or its rapid elimination from serum (19). In any case CSF sample would not be ideal for routine diagnosis of TBI.

1.3.2 Glial fibrillary acidic protein

Glial fibrillary acidic protein is a monomeric intermediate filament protein expressed by astrocytes and is released after head injury (23).

Glial fibrillary acidic protein is only found in the central nervous system and therefore highly specific for brain tissue. GFAP is also not affected by multiple injuries (25). In a prospective study (Vos et al 2004) of 85 patients with traumatic brain injury (TBI), admission serum levels of GFAP and neuron-specific enolase (NSE) combined with GCS gave a good
predictive model of outcome. Serum levels of GFAP greater than 1.5µg/l strongly predicted death. In the same study a serum S100B level of > 1.13µg/l was the strongest predictor of death (26). In another prospective study (Pelinka et al 2004) that enrolled 114 patients with TBI that aimed to determine whether GFAP was released after TBI and whether it was related to severity and outcome it was reported that GFAP was released in TBI and the release correlated with clinical severity and outcome. The study also showed that GFAP was not released after multiple injuries without TBI (27). Although GFAP is specific to the brain it has limited potential clinical utility because of none specific methodology (28).

1.3.3 Myelin basic protein

Myelin basic protein is major protein component of myelin that surrounds the axons of neurons (29). MBP is released into CSF and serum when there is injury to white matter and the levels remain elevated two weeks post injury (30). Serum MBP is also increased in de-myelinating disease (31). Since MBP is the main protein component of myelin any damage to the myelin sheath also cause damage to the blood-brain barrier resulting in entry of MBP and that of other CNS-derived biomarkers into the blood (18). Haemolysis causes an artificial increase of MBP. In a prospective case control study involving 100 patients (children) it was reported that MBP had the lowest sensitivity (44%) compared to NSE (71%) and S100B (77%) in identifying TBI (32). The methods used for MBP measurement lacks specificity and the marker has not therefore been adopted as a routine TBI biomarker (33).

1.3.4 Neuron-specific enolase

Neuron-specific enolase is an isoenzyme of the glycolytic enzyme enolase. It is found in brain, peripheral nervous tissue and other neuroendocrine tissue. In the brain NSE is expressed in cytoplasm of neurons, oligodendrocytes and neuroendocrine cells (34). It is also
found in neuroendocrine tumours, glucagonomas, insulinomas, carcinoid tumours of the intestine, neuroblastomas, thyroid medullary carcinoma and metastatic tumours. Neuron-specific enolase has been used as a marker for tumours of neuroendocrine origin and lung cancer (35). Neuron-specific enolase is the only marker that directly assesses functional damage to the neurons. It is not secreted by cells and therefore its passive release after cell destruction and its increased concentration in blood directly indicate neuronal structural damage (36). Erythrocytes contain large amount of NSE and therefore haemolysis greatly affects its measurement in blood (37).

Increased activity of NSE was reported in the serum of patients with stroke, intracerebral haemorrhage and after cardiopulmonary resuscitation (38). Increased activity of NSE has also been reported in patients with severe sepsis and septic shock and is attributed to brain injury (39). El-Maraghi et al (2013) reported that in traumatic brain injury high serum levels of NSE correlates with injury severity and clinical outcome (40). NSE activity increases in the first 12 hours after trauma and decreases within a period of hours to days. Manfred H et al (2003) reported a high significant correlation of serum NSE levels with lesion size in CT (41). Increased activity of NSE has been reported in patients with multiple injuries but without traumatic brain injury and therefore the biomarker has not been adopted as a specific marker for brain injury (42).

1.3.5 S100B

S100 proteins are group of calcium modulated proteins. The S100 proteins are so named because of their 100% solubility in a saturated solution of ammonium sulphate. These proteins were first identified by B.W Moore in 1965 (43).
1.3.5.1 Structure and function of S100B

S100 proteins are small acidic proteins of 10-12kDa which contain two distinct helix E-loop-helix F (EF hands), four \( \alpha \)-helical segments, a central hinge region of variable length and the N- and C- terminal domains. Each S100 polypeptide is composed of two EF- hands \( \text{Ca}^{2+} \)-binding domains connected by a central hinge region. The C-terminal EF-hand contains the classical \( \text{Ca}^{2+} \)-binding motif common to all EF-hand proteins and contains a typical sequence of 12 amino acids flanked by helices \( H_{111} \) and \( H_{1v} \). The N-terminal EF-hand is different from the classical EF-hand and is characteristic of the S100 proteins. The N-terminal EF-hand with a 14 amino acid sequence is flanked by helices \( H_1 \) and \( H_{11} \) and is called the S100 specific EF-hand (44).

S100 proteins usually exert their function through calcium binding although zinc and copper have also been shown to regulate their biological activity. When \( \text{Ca}^{2+} \) binds to S100 protein at the C-terminal there is a conformational change mainly due to reorientation of the \( H_{111} \) helix whereas the \( H_{1v} \) helix does not move. Minor structural changes take place at the N-terminal \( H_1 \) and \( H_{11} \) helices upon \( \text{Ca}^{2+} \)-binding. \( \text{Ca}^{2+} \)-induced conformational change at the C-terminal opens the structure and exposes a wide hydrophobic cleft formed by residues of \( H_{111} \) and the C-terminal loop. The hydrophobic cleft is the interaction site of S100 proteins and their target proteins (45). Twenty five proteins have been identified as belonging to the S100 family with twenty one of the proteins having genes located on the same chromosome loci.

The S100 family is composed of S100A1-S10018, trichohylin, fillagrin and repetin (located on chromosome 1q21), S100B (chromosome 21q22), S100G (chromosome xp22), S100P (chromosome 4p16) and S100Z on 5q14. S100B and S100A1 were the first proteins of the S100 family to be purified from bovine brain and were defined as brain specific (46). Within the cell some S100 proteins exist as homodimers of two monomers held together by non-
covalent bonds. S100A4, S100A6, S100A7, S100A8, S100A10 and S100A11 exists as homodimers. Some S100 proteins form heterodimers as the case with S100A1/S100B, S100A8/S100A9, S100B/S100A6, S100A1/S100A4 and S100B/S100A11 dimers (47).

Members of the S100 protein are multifunctional signalling proteins involved in the regulation of cellular processes such as contraction, motility, cell growth, differentiation, cell cycle progression, transcription and secretion. The variety of functions is as a result of the different members and is modulated by the different metal ion-binding capacity of the different members. In addition to their intracellular function, several S100 proteins such as S100B, S100A4, S100A8, S100A9, S100A12 and S100A13 act as cytokines. The heterodimer S100A8/A9 act as a chemotactic molecule in inflammation, S100B exhibits neurotrophic activity, S100A4 has angiogenic effects and S100A12 is involved in host-parasitic response (48).

1.3.5.2 Disease association

Diseases associated with altered expression levels of S100 proteins can be classified into four categories namely neoplastic disorders, cardiac diseases, inflammatory diseases and neurological diseases.

1.3.5.2.1 Neoplastic disorders

Different forms of cancer exhibit changes in the expression of S100 proteins.

Increased levels of S100A4 are associated with poor survival rates in breast cancer patients. In a study done by Ismail et al (2008) it was reported that there is increased expression of S100A4 protein in early and advanced breast cancer stages compared to the normal breast (48). Increased levels of S100A4 is also found in patients with oesophageal squamous, colon carcinoma, invasive pancreatic carcinoma and non small cell lung cancer and these
increases are associated with poor prognosis (49). S100B is secreted in malignant melanoma and serum levels of S100B have been used for establishing prognosis, evaluating treatment success and predicting relapse (50). S100B has also found application as a marker for detection of brain metastases and has a good negative predictive value compared to radiological investigations (51). Since S100B is also increased in cerebrovascular ischemic changes it is recommended that marker be measured in conjunction with pro-Apolipoprotein A1 for a sufficient specific serum based diagnosis of the presence of metastatic brain tumours (52).

1.3.5.2.2 Cardiac diseases

S100A1 is specific to the myocardium and is highly expressed in that tissue. S100A1 modulates contraction of the myocardium by releasing calcium from the sarcoplasmic reticulum through its interaction with ryanodine (53). S100A1 is up-regulated in right ventricular hypertrophy and down-regulated in end-stage heart failure (54).

1.3.5.2.3 Inflammatory diseases

S100A8, S100A9 and S100A12 are predominantly expressed in phagocytes and are associated with pro-inflammatory functions. High concentrations of these proteins prevail in inflammatory disorders such as rheumatoid arthritis, chronic bronchitis and cystic fibrosis (55).

1.3.5.2.4 Neurological diseases

S100B is primarily produced by astrocytes in the central nervous system and its increased expression indicates astrocytic activation. An immunohistochemical study identified astrocytes as the predominant S100B- positive cells in gray matter and oligodendrocytes as the predominant S100B- positive cells in white matter (56).
Secretion of S100B is an early indication of astrocytes response to metabolic injury caused by oxygen and glucose deprivation (55). Traumatic brain injury results in increased levels of S100B in cerebrospinal fluid and blood (57). Increased levels are also observed in Alzheimer’s disease, Down syndrome and multiple sclerosis (58). It has been reported that nanomolar levels of S100B in the extracellular space promote neuron survival and growth whereas increased micromolar concentration promote cell death (59). The biological half life of S100B in circulation is reported as between 30-60min. The protein is completely cleared from circulation through the kidneys within 2hours (60). Persistent elevations of S100B in serum reflect either a continued active secretion of the protein or passive release from damaged tissue (61). In a review article done by Linda E Pelinka (2004) it was reported that in patients with TBI without multiple trauma serum S100B levels measured within 24 hours post trauma gave a good prediction of outcome. Serum S100B levels of survivors returned to normal 48 hours post trauma (62).

In a study carried out by Borg et al (2012) comparing three markers of brain injury S100B, neurone specific enolase (NSE) and myelin basic protein (MBP) it was reported that S100B was the better marker of the three in predicting the outcome of both mild and severe head injury subjects. The primary outcome was the baseline measurement of the three markers. CT scan results were used as the second measure of outcome. CT scan results were classified as positive if at least one of the following evidence was demonstrated: subdural haematoma, epidural haematoma, subarachnoid haemorrhage, cerebral contusion or diffuse axonal injury. The final measure of outcome was done by making a follow up on patients and ascertaining whether they had returned to normal daily activities two weeks after injury (63).
Gianfranco Cervellin et al (2012) compared the levels of S100B and CT scan findings in 60 participants and reported that S100B levels were higher in patients with positive CT scans than in patients with negative CT scan (1.35 versus 0.48 µg/L; p value <0.001).

Using a cut-off of 0.38µg/L S100B displayed a sensitivity of 100% and specificity of 58% (64). Biberthaler et al (2006) reported that using a cut-off of 0.10µg/L for serum S100B helped identify patients with head trauma and this was correlated with lesions on CT scan with a sensitivity of 99% and specificity of 30%. This finding indicated that if measurement of S100B concentrations are considered in clinical decisions scans can be reduced by 30% (65). Calcagnile et al (2012) also reported that S100B had a sensitivity of 100% and specificity of 28% for identifying intracranial complications (66).

Another review done by Kovesdi et al (2009) summarised protein biomarkers in mild and severe TBI in adults and children, and concluded that only S100B consistently predicted injury severity and outcome (67). Kofias et al (2007) reported that S100B protein reflects injury severity and improves outcome prediction in severe head injury. In the same study it was also reported that S100B can also be used to assess efficacy of treatment in the same group of patients (68). Townend and Igebrigtsen (2006) in another review of published literature on the role of S100B protein in head injury prediction reported that patients with high levels of S100B (>2.5µg/l) at the initial assessment were at high risk of disability after head trauma (69).

Thelin et al (2013) in a study reported that serum S100B levels sampled within 12-36 h gave a better prediction of outcome than when sampled within the first 12 h (70).

Kleindienst et al (2006) suggested that serum and S100B levels are poorly correlated as serum levels depends primarily on the integrity of the blood brain barrier and not on the level
of S100B in brain. They further suggested that because of the neurotrophic effect of S100B, the protein contributes to neuronal repair thereby reducing neuronal injury. Because of the therapeutic value of S100B, it is more useful when used to improve outcome in patients who sustain head injury than when used as a negative determinant of outcome in TBI (71).

Muller et al (2007) in a study were they compared CT scan results and serum S100B levels in patients with minor head injury determined that measurement of serum S100B levels cannot replace CT scanning but adding S100B measurement to the clinical evaluation could only help in selecting patients for CT scanning (72).

Routsi et al (2006) in a study done on critically ill patients admitted in intensive care, without evidence of brain injury or any neurological disorder, reported that all patients exhibited increased serum levels of S100B (median, 0.31µg/l) at least once (73). There are also controversies on whether serum S100B levels are increased in extra-cranial injuries. Pham et al (2010) reported that S100B levels are not increased in extra-cranial injuries (74) whereas Savola et al (2004) reported that large extra-cranial injuries caused increased serum S100B levels (0.35µg/l) (75). Although serum S100B levels were increased in extra cranial tissue Savola et al demonstrated that because of the high negative predictive value for S100B normal values of S100B obtained shortly after trauma excluded brain injury with high accuracy (75).
1.4 STUDY JUSTIFICATION

Many patients with head injuries in public health institutions in Zimbabwe face challenges of payment for CT scans whose cost is way above the reach of most. An alternative quantitative biomarker if validated for clinical usefulness, may improve the diagnosis and care of head injury patients. In the present study the clinical utility of S100B as a biomarker for head injury was evaluated.

1.5 RESEARCH QUESTIONS

1. What is the correlation, if any between serum S100B levels and CT scan results of TBI patients admitted to Parirenyatwa hospital neurological ward?

2. What is the correlation, if any between serum S100B levels and GCS in patients with TBI admitted to Parirenyatwa hospital neurological ward?

3. Can serum S100B levels predict the outcome (GOS) in patients with TBI admitted to Parirenyatwa hospital neurological ward?

1.6 HYPOTHESIS

H₀ There is no correlation between serum S100B levels and positive CT scan findings or GCS in patients admitted to hospital with traumatic brain injury.

H₁ Serum S100B levels are significantly correlated with positive CT scans in patients admitted to hospital with traumatic brain injury.

1.7 OBJECTIVE

To determine the strength of the correlation between CT scan findings and serum S100B levels in patients with head injuries.
1.7.1 Specific objectives:

1. To determine the levels of S100B in apparently healthy individuals.

2. To determine the levels of S100B in patients with non-neurological medical conditions.

3. To determine the levels of S100B in patients with head injuries.

4. To determine the correlation (if any) between S100B and CT scan findings.

5. To determine the association (if any) between S100B levels and severity of injury and also with outcome in patients with head injuries.
CHAPTER 2

2.0 MATERIALS AND METHOD

2.1 Study design

In this analytical cross sectional study patients with head injuries were recruited from B9, a neurological ward at Parirenyatwa hospital, Harare, Zimbabwe from November 2012 to May 2013. Only patients who gave written informed consent were enrolled in this study. For the patients who were unconscious consent was sought and granted from the legal representatives. The study commenced after ethical approval was sought and granted by the institutional committees Joint Research Ethics Committee (JREC) and the Medical Research Council of Zimbabwe (MRCZ).

Fifty blood samples for the determination of S100B levels were collected from patients within 24hrs after admission into the neurological ward B9 at Parirenyatwa Hospital. Head injury audit reports were accessed to ascertain GCS, GOS and CT scan findings. Serum S100B levels were correlated to CT scan findings, Glasgow coma scale and Glasgow outcome scale. Additional blood samples were collected from 20 apparently healthy consenting health workers and 20 left over samples were collected from Parirenyatwa Hospital Biochemistry Laboratory from patients admitted in medical wards with non-neurological conditions. Permission to collect left over samples was granted by the Parirenyatwa Hospital Clinical Director and the Head of Department in the Biochemistry Laboratory. These 40 samples comprised the comparison group.

After blood sample collection from both patients and apparently health controls the blood was allowed to clot and serum was harvested after centrifuging at 3000rpm for five minutes.
The specimens were stored at -70°C until time of analysis. All the samples were only thawed once just before analysis.

2.2 Study setting

2.2.1 Reference population

The reference population are all patients with traumatic head injury.

2.2.2 Source population

The source population were all patients presenting at Parirenyatwa Hospital with suspected head injuries.

2.2.3 Sampling frame

The sampling frame included patients with head injury admitted in ward B9 at Parirenyatwa hospital during the enrolment period who met the inclusion criteria.

2.2.4 Study population

The study population were patients admitted into ward B9 with head injury who consented to participate in the study, apparently healthy hospital workers and non-neurological patients admitted in medical wards at Parirenyatwa Hospital.

2.2.5 Study participants

The study participants included consenting patients aged 18 years and above admitted to ward B9 Parirenyatwa Hospital with head injury.
2.2.6 Case definition

Patients admitted to Ward B9 with traumatic head injury and a Glasgow coma scale between 3-15.

2.2.7 Control definition

Two sets of controls were included. These were apparently healthy consenting hospital personnel and patients admitted in adult medical wards who had non-neurological conditions.

2.3 Inclusion and exclusion criteria

2.3.1 Inclusion criteria

Participants were included in the study if they met the following criteria:

- Patients aged 18 years and above admitted in ward B9 with query traumatic head injury.
- Patients whose samples were collected within 24 hours of admission in ward B9 neurological ward.
- Patients or their legal representatives who consented to the study

2.3.2 Exclusion criteria

Patients were excluded from the study if:

- Aged below 18 years
- They had multiple injuries
- They did not consent to the study
2.4 Study factor

The study factors were serum S100B levels, CT, GCS and GOS.

2.5 Outcome factor

The outcome factor was serum S100B levels correlation with CT findings, GCS and GOS.

2.6 Sample size

The sample size calculated based on the study by Figueiredo et al 2006 (76) in which a mean serum concentration for S100B of 0.29μg/L was reported in 44 patients with mild head injury and 0.04μg/L in 21 apparent healthy controls. Assuming a common standard deviation in the mean of S100B serum levels to be 0.28μg/L, the minimum required sample size was calculated as:

\[ n = \frac{2(Z_{\alpha/2} + Z_\beta)^2}{\Delta^2} \]

where \( \Delta = (\mu_1 - \mu_2)/\sigma \) is the effect size and \( Z_\beta = 0.84 \) corresponds to 80% power.

The minimum required sample size of each group of patients was calculated to be 20 participants. The sample size for the present study included blood samples from 50 patients with head injuries, 20 serum samples from apparently healthy hospital personnel and 20 leftover serum samples from non-neurological patients admitted in medical wards.

2.7 Ethical considerations

Ethical approval was sought and granted by the Joint Research Ethics Committee (JREC) of the University of Zimbabwe and Parirenyatwa Hospital and from the Medical Research Council of Zimbabwe (MRCZ).
2.8 Blood Sample collection

Five millilitres of whole blood was collected by venipuncture from each volunteer and aliquoted into a plain tube. The blood samples were left to clot at room temperature and serum harvested after centrifuging for 5 minutes at 3000rpm. The serum samples were kept at -70°C until time of analysis. Blood samples collected from apparently healthy individuals were treated in the same manner as case samples and also kept at -70°C until time of analysis. Left over samples from patients admitted in medical wards were collected from the biochemistry laboratory at Parirenyatwa Hospital on the day the samples were collected and stored at -70°C until time of analysis.

2.9 Laboratory methods

Serum S100B levels were measured using an enzyme- linked immunoabsorbent (ELISA) technique when the required sample size for the case participants and controls was achieved. All serum samples and procedural controls were assayed in duplicate. The S100B (human) ELISA kit (BOBV01090J00022 REF KA0037 LOT E12-106) used was manufactured by Abnova Company, Jhongl City, Taiwan.

The assays were carried out at Premier Clinical Laboratory, Harare, Zimbabwe on an automated ELISA platform, HUMAN Elisys Uno HumaReader manufactured by Gesellschaft fur Biochemica und Diagnostica mbH, Wiesbaden, Germany.

2.9.1 Principle of test

In an Enzyme-linked immunoabsorbent technique reaction one of the reaction components is attached to the surface of a solid phase, in the current study micro titre wells. The antibodies in the solid phase then bind to the antigen (S100B) in calibrators, controls and serum when aliquots are added to the micro titre wells. After washing an enzyme- labelled antibody
different from the one in the solid phase is added to form a sandwich (antibody-antigen-enzyme-labeled antibody). Excess unlabelled antibodies are removed by washing and then the enzyme substrate is added. The amount of product produced by the enzyme catalysis is proportional to the quantity of antigen in the sample (77).

2.9.2 S100B measurement

S100B was measured using a sandwich ELISA technique on an automated ELISA platform. Specifically standards, quality control material and study serum samples were incubated at room temperature (25°C) in micro plate wells pre-coated with polyclonal anti-bovine S100B antibody for 120 minutes. After incubation and washing, biotin-labeled monoclonal anti-human S100B antibody was added to the wells and incubated at room temperature with captured S100B for 60 minutes. Streptavidin-Horse Radish Peroxidase conjugate was added after a second washing and incubated at room temperature for 30 minutes. After a third wash the conjugate was allowed to react with a substrate solution, tetramethylbenzidine (TMB) at room temperature for 15 minutes. The reaction was stopped using molar sulphuric acid and the absorbance of the final solution was measured at 450 nm within 5 minutes of reaction stoppage. Results of S100B concentration were extrapolated from a standard curve.

2.9.3 Performance Characteristics

The S100B kit manufacturer cites an assay linearity of 15-2000pg/ml. Dilution and reanalysis were recommended for serum sample readings above 2000pg/ml. The method has a reported intra-assay coefficient of variation of 10.1%. The antibodies used in the assay are reported to be highly specific to human S100B and showed no cross reactivity with other S100 proteins.
2.9.4 Assay modification

The lowest concentration of standard prepared according to manufacturer’s instruction was 50pg/ml and the detection limit of assay cited was 15pg/ml.

To lower the detection limit a serial dilution was done on the 50pg/ml to obtain 25pg/ml and 12.5pg/ml concentrations. All the samples that had S100B concentrations of <15pg/ml were re-assayed using a new calibration curve including the 25 and 12.5pg/ml concentrations to get absolute values.

2.10 Data management and Statistical analysis

2.10.1 Data management

Consenting participants were assigned a unique identifier number which was also used to identify blood samples. Patient hospital numbers were used to access head injury audit reports. CT scan results, GCS and GOS were obtained from the audit reports. The audit forms were completed by doctors on patient discharge or death. The CT scan results were considered positive when one or more of the following diagnosis was made: head injury with diffuse axonal insult, brain contusions, epidural haematoma, subdural haematoma, depressed skull fracture, linear fracture or base of skull fracture. CT scan results were considered negative when none of the above diagnosis was made. The GCS used the classification system of clinical severity in TBI with a scale of 3-15. The GOS score was determined by the clinician on patient discharge or death. The data was kept under lock and key by the investigator and was only accessible to members of the research team.
2.10.2 Statistical analysis

Data were entered in Microsoft excel and converted into STATA Version 13 for cleaning and analysis.

Baseline demographic characteristics were presented using median and IQR for continuous variable such as age because data was not normally distributed and percentages for categorical variables such as sex. A histogram of causes of head injury was presented. Levels of S100B for different types of participants were presented using medians and IQR. One way analysis of variance (ANOVA) was used to compare S100B levels among the different types of participants. A Bonferroni post test was done to show where significant differences in S100B lie among these participants. Kruskal-Wallis test was used to compare differences in S100B levels between participants diagnosed as negative and those diagnosed as positive by CT scan. We also presented a table showing patient outcome and median S100B values. The sensitivity and specificity of S100B in head injury were also determined. A p < 0.05 was taken to be significant.
CHAPTER 3

RESULTS

3.1 Participants Baseline Data and Serum S100B levels

Table 1: Baseline demographic characteristics of study participants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All</th>
<th>AHP</th>
<th>NNHP</th>
<th>HIP</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age Median(IQR)</td>
<td>33.0(28-43)</td>
<td>30.5(27-44.5)</td>
<td>32.5(28-46.5)</td>
<td>33.5(29-40)</td>
<td>P=0.887</td>
</tr>
<tr>
<td>Sex [n (%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>62(69%)</td>
<td>8(40%)</td>
<td>12(60%)</td>
<td>42(84%)</td>
<td>p =0.001</td>
</tr>
<tr>
<td>Female</td>
<td>28(31%)</td>
<td>12(60%)</td>
<td>8(40%)</td>
<td>8(16%)</td>
<td></td>
</tr>
<tr>
<td>S100B units Median(IQR)</td>
<td>18.2(9.4-183.9)</td>
<td>8.3(5.25-9.8)</td>
<td>12.75(9.75-18.2)</td>
<td>173.25(50-428.1)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>


A total of 90 consecutive participants comprising 20 apparently healthy individuals, 20 participants admitted into hospital with non-neurological conditions and 50 patients with acute head injuries were enrolled into the study as shown in Table 1. The median age of the participants was 33 years (IQR 28-43). The median age for apparently healthy participants (30.5 years) was slightly lower but not significantly so than that of the other two categories (p=0.887). Males comprised 84% of the patients with acute head injuries. Males were more represented than female patients (p=0.001). Overall for all participants males comprised 69% of the study participants.
Median (IQR) S100B levels among apparently healthy participants, participants with non-neurological condition and participants with head injuries were 8.3(5.25-9.8), 12.75(9.75-18.2) and 173.25(50-428.1) respectively. S100B levels among participants with head injuries were statistically significantly higher compared to apparently healthy participants and participants with non-neurological condition (p<0.001).
3.2 Causes of Head Injury

Figure 1: Causes of Head Injury

Figure 1 shows the causes of injury in traumatic brain injury suspects admitted to ward B9 at Parirenyatwa Hospital in Harare during the study period. Of the 50 patients, 39 (78%) of the patients had head injury as a result of motor vehicle accident (MVA), 8(16%) of the patients had head injury as a result of assault, 2(4%) of the patients had head injury as a result of a fall and 1(2%) of the patients had head injury as a result of being hit by a falling tree.
3.3 CT Scan Findings

Of the 50 head injury suspects admitted to hospital 29 had a scan done as part of routine care. The remainder did not undergo the procedure because they could not afford the cost of the procedure. CT scan findings were positive for 25 patients and negative for 4 patients. The median (IQR) for serum S100B levels was 10.5 (7.0-14.0) for the CT negative group and 174.0 (100.4-298.0) for those with a positive CT scan. Median serum S100B levels were statistically significantly higher in patients with positive CT scan compared to those with negative CT findings (p =0.003).
3.4 Sensitivity and Specificity of Serum S100B as a Biomarker for Head Injury

The sensitivity and specificity of S100B as a biomarker of traumatic head injury was determined at cut off points of 40pg/ml, 50pg/ml and 100pg/ml. In all instances CT scan findings were adopted as the gold standard diagnostic tool. Below are presented calculated sensitivities and specificities.

Table 2: Sensitivity and Specificity of S100B at three Cut-off Points

<table>
<thead>
<tr>
<th>Cut-off (pg/ml)</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>88</td>
<td>100</td>
</tr>
<tr>
<td>50</td>
<td>84</td>
<td>100</td>
</tr>
<tr>
<td>100</td>
<td>80</td>
<td>100</td>
</tr>
</tbody>
</table>

The serum S100B had a specificity of 100% at all the three cut off points with the sensitivity ranging from 88%, 84% and 80% for cut off points of 40pg/ml, 50pg/ml and 100pg/ml respectively.
3.5 Admission Glasgow Coma Scale Scores

The 50 patient admitted with suspected traumatic brain injury had Glasgow Coma Scale scores evaluated on admission. Total scores are restrained between 5-15 points. Five (10%) patients had a total score corresponding to severe head injury, 22 (44%) were classified as having moderate head injury and 23 (46%) were classified as having mild head injury. These three categories corresponded to median (IQR) S100B levels of 400.3 (274.7-432.5), 299.6 (154.1-542.6) and 51.8 (13.0-172.3) respectively. Patients with moderate and those with severe head injuries had statistically significantly higher median S100B levels compared to those with mild head injuries. (p<0.001)

Figure 2: Correlation between Serum S100B and Glasgow Coma Scale Scores

Serum S100B levels were moderately negatively correlated with the Glasgow Coma Scale scores. (r = -0.39)
3.6 Glasgow outcome score at hospital discharge

Table 3: Baseline Serum S100B levels and status of Participant on Hospital Discharge (GOS)

<table>
<thead>
<tr>
<th>Grade</th>
<th>Frequency n (%)</th>
<th>S100B Median (IQR)</th>
<th>Positive CT n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death</td>
<td>12 (24%)</td>
<td>442.1 (337.5-621.5)</td>
<td>5 (20%)</td>
</tr>
<tr>
<td>Vegetative state</td>
<td>0 (0%)</td>
<td>-</td>
<td>0%</td>
</tr>
<tr>
<td>Severely disabled</td>
<td>4 (8%)</td>
<td>211.5 (106.65-341.6)</td>
<td>4 (16%)</td>
</tr>
<tr>
<td>Moderately disabled</td>
<td>1 (2%)</td>
<td>14 (0)</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Good recovery</td>
<td>33 (66%)</td>
<td>100.4 (14.0-238.15)</td>
<td>15 (60%)</td>
</tr>
</tbody>
</table>

The median (IQR) S100B levels for participants who demised and those who survived was 442.1 (337.5-621.5) and 110.3 (19.0-250.7) respectively. The difference was statistically significantly (p<0.001). Of those that died 5 had positive CT scans.
CHAPTER 4

4.0 DISCUSSION AND CONCLUSION

4.1 DISCUSSION

The main objective of the present study was to evaluate the clinical utility of serum S100B levels in the diagnosis of traumatic brain injury patients. The present study was stimulated by the high cost of imaging procedures in Zimbabwe and a need to adopt a cheaper surrogate biomarker. In order to achieve that objective, 50 traumatic brain injury suspects were enrolled on admission into a neurological ward at Parirenyatwa Hospital, Harare, Zimbabwe. Serum S100B levels were measured and out of these, 29 patients had CT scans done. CT scans were not done on the other 21 patients because they could not afford the services. Serum S100B levels were also determined in 20 apparently healthy individuals and 20 patients admitted into the same hospital with non-neurological complaints.

Scandanavians guidelines for management of mild head injury recommend CT scans for all patients with GCS of 14-15, loss of consciousness and /or amnesia. Where CT scan is not available, hospital admission with observation is recommended. CT scan is also recommended for mild patients with clinical signs of depressed skull or signs of clinical deterioration on hospital admission (78). The present study was done at a hospital where similar guidelines are adopted. S100B has been introduced as a clinical tool in emergency department at Halmstad, Sweden and all patients with levels less than 100pg/ml are recommended for discharge without further investigation (66). Calcagnile et al (2012) reported S100B sensitivity of 100% and a specificity of 28% for identifying intra cranial injuries (66).
The results indicate that 39(78\%) of the head injuries occurred as a result of motor vehicle accidents. This finding is in keeping with reports by Madikians A and Giza C (2006) who reported motor vehicle accidents as the main cause of TBI in developing countries (2).

In this study serum S100B levels in head injury patients were much higher in head injury patients compared to apparently healthy participants and participants with non-neurological diseases (p <0.001). This finding was similar with the findings of Savola et al (2004) where he reported increased S100B levels in patients with head injuries compared to those with extra cranial injuries and normal participants. In the same study he reported that S100B levels correlated with the severity of brain injury with the values around 1270pg/ml being observed in patients with moderate and severe head injuries (75).

The median S100B level in apparently healthy participants in this study was 8.3pg/ml (5.25-9.8). Literature reports different S100B levels for apparently healthy individuals. Anczykowski et al (2012) reported undetectable S100B levels in normal individuals using electrochemiluminescence immunoassays (ECLIA) on Cobas 6000 analyser (Roche Diagnostics) (79). Poli de Figueiredo et al (2006) reported the median of S100B levels in 21 healthy volunteers as 0.04µg/l (40pg/ml) using a newly developed heterogeneous immunoassay (Elecsys 2010) (76). Borg et al (2012) reported a 98th percentile reference limit for serum S100B of 21pg/ml in adult healthy controls using a manual ELISA method (63). Commercial kits generally gave high values of S100B (as high as 100pg/ml) compared to the manual ELISA methods (80). This study also showed that S100B only increased in head injury and not in non-neurological diseases (p value <0.001). This wide variation in reported serum S100B levels in apparently healthy volunteers probably justifies for standardisation of S100B assays to allow for harmonisation of results using different assays.
There were statistically significant differences in serum S100B levels between participants diagnosed as negative and those diagnosed as positive by CT scan (p = 0.003). In this study all patients who had negative CT scans also had low serum S100B levels (<15pg/ml). Unden and Rommer (2010) in a meta-analysis reviewed 12 studies that consistently showed the ability of serum S100B to predict normal CT scan (81). S100B has a short biological life and therefore timing of specimen collection is important as reported by Jackson et al (2000). Thus, in order to get useful diagnostic information, sampling should be done as early as possible (82). In all the studies specimens were collected within 3-24 hours post injury (81). In the current study specimens were collected within 24 hours of patient admission in ward without considering time of injury as this information was missing in audit reports.

In the current study the sensitivity and specificity of S100B for head injury was determined using 3 different cut-offs (40, 50 and 100 pg/ml). All 3 cut-offs gave a specificity of 100% then sensitivities of 88% (40pg/ml), 84% (50pg/ml) and 80% (100pg/ml). The meta-analysis by Unden and Rommer (2010) showed high sensitivities (range 75-100%) for S100B and specificities that ranged from 28-77%. The lowest cut-off used in these studies was 0.10µg/l (100pg/ml). Nygren de Boussard et al (2004) collected blood samples within 24 hours post injury and reported the sensitivity of S100B to be 80% at 0.15µg/l (150pg/ml) cut-off similar to the one obtained in this study at 100pg/ml cut-off. The sample size (29 participants) that was used to determine the sensitivities and specificities of S100B for head injury in this study was probably too small and further studies with larger sample sizes are recommended. The present study did not assess correlations of S100B levels with defined pathological states obtained by CT scanning. Unden et al (2005) however, reported correlation between serum S100B concentrations and epidural haematomas (83). Future studies can be done to reassess the correlation between serum S100B levels and CT scan diagnosis.
Ruan et al (2009) reported that using S100B as a screening tool in mild head injuries instead of CT scanning lowered costs because of the less time required for blood tests and also to avoid the increased rates for CT scans (84). Most laboratories in Zimbabwe can process S100B using automated immunoassays in 30min-1 hour. Biberthaler et al (2006) reported an automated device that can process serum S100B levels in 18 minutes (65). Public health institutions in Zimbabwe have perennial challenges with resources for CT scans and most patients requiring that service are usually referred to private institutions with costs ranging from US 150-500 dollars. These charges are beyond the reach of many patients. Introduction of serum S100B on the test menu of clinical laboratories as an initial screening tool can substantially reduce unnecessary CT scans and reduce hospital stay.

The highest number of patients (46%) in this study had mild head injury as defined by GCS. The median S100B level in the mild head injury group of 51.8pg/ml (13.0-172.3) was observed compared to those with moderate and severe head injury whose median S100B levels were 299.6 pg/ml (154.1-542.6) and 400.3 pg/ml (274.7-432.5) respectively. There was a statistically significant difference between serum S100B levels in patients with mild head injuries than those with moderate and severe head injuries (p <0.001). These findings were consistent with the findings of Savola et al (2004) who reported that S100B correlated strongly with the severity of brain injury (75). The current study showed a negative correlation (r = -0.39) between serum S100B levels and GCS. Anczykowski et al (2012) reported a negative correlation (r = -0.69) between serum S100B levels and GCS in patients with haematomas (79). There was not much difference between the median of the moderate group compared to the severe head injury group this was probably because of the small sample size (n=5) in severe head injury group.
The Glasgow outcome scale was determined at the point of hospital discharge or death. This was done from day 1 to day 21 depending on patient’s stay in hospital. During the period of this study Parirenyatwa hospital was the only referral hospital for neurological patients in Zimbabwe and therefore patients were discharged or referred back to other hospitals when they had stabilised to create room for other patients. No follow up was done by the researcher on patients after discharge from the referral centre. Higher serum S100B levels [median 442.1 pg/ml (IQR 337.5-621.5)] were found in patients who demised compared to those who survived [median 110.3pg/ml (IQR 19.0-250.7)]. This was consistent with the findings of da Rocha et al (2006) who reported increased levels of S100B levels of 2100pg/ml in patients with fatal outcomes compared with S100B levels (mean 850pg/ml) in patients who survived (85).

Although GOS was assessed during a short period and there was no patient follow up, results in the current study clearly indicated that serum S100B levels (median 100.4pg/ml, IQR 14.0-238.15) in patients with good recovery were lower compared to the poor outcome groups (severely disabled group, median 211.5 pg/ml IQR106.65-341.6 and death, median 442.1 pg/ml IQR 337.5-621.5).The results obtained in the current study agreed with the results obtained by Rothoerl et al (2000) who reported that patients with good recovery after 3 months had lower serum levels of S100B on admission compared to patients with poor outcomes (86).

4.2 CONCLUSION

The current study demonstrated that serum S100B levels have the ability to predict CT scan results. There was a correlation between serum S100B levels and clinical severity (GCS) and outcome (GOS) in patients with head injury. If serum S100B is introduced as a clinical tool in the emergency department the number of patients that require CT scanning can be reduced.
Serum S100B levels can also be measured in patients who cannot afford CT scans and this can help in patient management. Further studies that include proper sampling of samples within 3-24hrs post injury can be done to correlate serum S100B levels to pathological states so that patients who cannot afford CT scans but need surgical intervention can be identified using S100B levels.
REFERENCES


32. Berger R P, Adelson P D, Pierce M C, Dulani T, Cassidy L D, Kochanek P M. Serum neuron-specific enolase, S100B and myelin basic protein concentrations after inflicted


44. Marenholz I, Heizmann C W, Fritz G. S100 proteins in mouse and man: from evolution to function and pathology (including an update of the nomenclature). *Biochemical and Biophysical Research Communications* 2004; 322: 1111-1122.


61. Ana Rodríguez-Rodríguez, Juan José Egea-Guerrero, Antonio León-Justel, Elena Gordillo-Escobar, Jaume Revuelto-Rey, Ángel Vilches-Arenas, Antonio Carrillo-Vico, José María Domínguez-Roldán, Francisco Murillo-Cabezas, Juan Miguel Guerrero. Role of S100B protein in urine and serum as an early predictor of mortality


86. Rothoerl R D, Wortgen C, Brawanski A. S100 serum levels and outcome after severe head injury. *Acta Neurochirurgica* 2000; 76-100 (Supplement).
SUBJECT INFORMED CONSENT

PROTOCOL TITLE: Serum S100B level measurement as a biomarker of head injury.

DETAILS OF RESEARCHER: My name is Immacullata Makanza. I am studying for an MSc in Clinical Biochemistry at the University of Zimbabwe with the Institute of Continuing Health Education. As part of my study I am required to submit a research project.

PROJECT DESCRIPTION: I am conducting this research to evaluate the clinical utility of S100B as a biomarker of head injury. The study also aims to compare if there is any correlation between serum S100B levels and CT scan findings. Lastly the study aims to assess if S100B levels can be correlated to outcome and or duration of stay in hospital for head injury patients.

YOUR RIGHTS: Before you decide whether or not to volunteer for this study you must understand its purpose, how it may help you, any adverse effects and what is expected of you. This process is called informed consent.

PURPOSE OF RESEARCH: The purpose of this research is to find out the relationship between serum S100B levels and CT scan findings.

PROCEDURES INVOLVED IN THIS STUDY: I am going to access your patient records to find out the nature of head injury and time when the injury occurred. A sample is going to be collected as routine tests are done on your admission into the ward for the measurement of S100B protein (S100B protein is released into serum after brain trauma). Your patient records are also going to be accessed to find out the CT scan findings. For unconscious patients consent will be sought from a legal representative.

CONFIDENTIALITY OF RECORDS: There shall be no records kept together of your data and your name so no one will know your results without your consent. You shall be assigned a study number which cannot be linked to your personal information by third parties. All data shall be kept under lock and key in the University of Zimbabwe department of Chemical Pathology for 3 years after which they shall be destroyed.

STUDY WITHDRAWAL: You can choose not to enter the study or withdraw from the study at any time without prejudice, loss of treatment or victimisation of any kind.

PROBLEMS/QUESTIONS: Please ask any questions or raise any queries you might have about this study through the investigator I.Makanza telephone 0773196156 or 04-705056-8.

AUTHORISATION: I have read and understood this paper about this study or it was read to me. I have understood that being in this study is voluntary and i may opt out at any time. I will get a copy of this consent form.
CHIVUMIRANO CHEKUPINDA MUTSVAGIRIDZO

MUSORO WETSVAGIRIDZO

Ongororo yeS100B muropa revanhu vakuvura musoro.

ZVAMUNGADE KUZIVA PAMUSORO PEMUNHU ARI KUITA TSVAGIRIDZO

Zita rangu ndinonzi Immacullata Makanza. Nditori mudzidzi weMSc yeClinical Chemistry pachikoro cheUniversity yeZimbabwe. Ndinotarisirwa kuti ndiite tsvagiridzo sechikamu chedzidzo yangu.

TSANANGURO PAMUSORO PETSVAGIRIDZO

Muongororo iyi nditori kutsvaga kuti ndione kuti S100B ye muropa ingashandiswa here kutaridza makuvariro emusoro anenge aitika mumunhu. CT scan ndiyo inoshandiswa nemachiremba kuona makuvariro anenge aita munhu musoro saka muongororo iyi ndiri kuda kuona kuti zvinovaniwaka nekushandisa CT scan zvinoenderana here nezvinovaniwaka nekuongorora S100B muropa. Chekupedzisira ndiri kuda kuona kuti huwandu hweS100B muropa hunobatsira here kutaridza kuti munhu anogona kupora here kana kuti achagara kwenguva yakadii muchipatara.

KODZERO YAKO

Usati wapinda mutsvagiridzo iyi unofanira kunzwisisa kuti iri kuitirwei, ingakubatsirei, pane njodzi ingakuwirei here uyezve chii chinotarisirwa kubva kwauri. Kana munhu abvuma kupinda mutsvagiridzo nemuitiro uyu ndiyo inonzi chivumirano chine kunzwisisa.

MURWERE ASINGAKWANISE KUZVIMIRIRA

Kune murwere arikurwarisa zvekuti haakwanise kuita sarudzo ega mvumo inotsvagwa kune munhu anomumirira zviri pamutemo.

CHINANGWA CHETSVAGIRIDZO

Nditori kuongorora kuti huwandu hweS100B muropa hunoenderana here nezvinobuda muCT scan.

NZIRA DZEKUONGORORA

Nevuvi yenyu ndichashandisa nhoroondo ye hurwere inenge yakanyorwa nachiremba uyezve ndichatarisa zvinenge zvabuda muongororo yeCT scan. Pamunotorwa ropa richiongororwa nana chiremba muchipihwa mubhedha, ndichatorawo ropa ndoongorora huwandu hweS100B huri muropa renyu.
CHITSIDZO CHEMAGWARO ENYU


KUBUDA MUTSVAGIRIDZO

Munokwanisa kuramba kupinda mutsvagiridzo kana kubuda chero ipi nguva zvisingakanganise mabatirwo enyu kana marapirwo enyu muchipatara.

MATAMBUDZIKO /MIVUNZO

Kana muine mibvunzo munogona kubvunza munhu ari kuita tsvagiridzo iyi iko zvino kana kumubata panhamba dzerunhare dzinoti 0773196156 kana pa04-705056-8.

MVUMO YENYU

Zita

Runyoro

Mumiririri

Runyoro

Runyoro rwemuongorori
Ref: MRCZ/B/398

Immaculata Makanza
University of Zimbabwe
Department of Chemical Pathology
P.O Box A178
Avondale
Harare

RE: SERUM S100B LEVEL MEASUREMENT AS A BIOMARKER IN PATIENTS WITH HEAD INJURY

Thank you for the above titled proposal that you submitted to the Medical Research Council of Zimbabwe (MRCZ) for review. Please be advised that the Medical Research Council of Zimbabwe has reviewed and approved your application to conduct the above titled study. This is based on the following documents that were submitted to the MRCZ for review.

a) Research proposal and summary
b) Informed Consent Form (English and Shona)

- APPROVAL NUMBER: MRCZ/B/398
  This number should be used on all correspondence, consent forms and documents as appropriate.

- APPROVAL DATE: 15 October 2012
- TYPE OF MEETING: Expedited
- EXPIRATION DATE: 14 October 2013

After this date, this project may only continue upon renewal. For purposes of renewal, a progress report on a standard form obtainable from the MRCZ website or our website should be submitted three months before the expiration date for continuing review.

- SERIOUS ADVERSE EVENT REPORTING: All serious problems having to do with subject safety must be reported to the Institutional Ethical Review Committee (IERC) as well as the MRCZ within 3 working days using standard forms obtainable from the MRCZ website: www.mrcz.org.zw
- MODIFICATIONS: Prior MRCZ and IERC approval using standard forms obtainable from the MRCZ website is required before implementing any changes in the Protocol (including changes in the consent documents).
- TERMINATION OF STUDY: On termination of a study, a report has to be submitted to the MRCZ using standard forms obtainable from the MRCZ website.
- QUESTIONS: Please contact the MRCZ on Telephone No. (04) 791792, 791193 or by e-mail on mrcz@mrcz.org.zw

Other:
- Please be reminded to send in copies of your final research results for our records as well as for the Health Research Database
- You are also encouraged to submit electronic copies of your publications in peer-reviewed journals that may emanate from this study.

Yours Faithfully

MRCZ SECRETARIAT
FOR CHAIRPERSON
MEDICAL RESEARCH COUNCIL OF ZIMBABWE

PROMOTING THE ETHICAL CONDUCT OF HEALTH RESEARCH
UNIVERSITY OF ZIMBABWE
COLLEGE OF HEALTH SCIENCES
MEMORANDUM

FROM: Chairman, Joint Research Ethics Committee  
TO: Immaculata Makanza, Department of Chemical Pathology  
c.c: The Chairperson, Department of Chemical Pathology

DATE: 27 Sept 2012
EXT: 2241/2242

RE: EVALUATION OF SERUM S100B PROTEIN AS A BIOMARKER OF HEAD INJURY IN PATIENTS ADMITTED IN A NEUROLOGICAL WARD B9 AT PARIRENYATWA HOSPITAL: JREC/242/12

Thank you for your application with the above mentioned title seeking approval from the Joint Parirenyatwa Hospital and College of Health Sciences Research Committee (JREC). The Committee has successfully evaluated and discussed the material you supplied.

It was agreed that your application be approved as a research project which is ethically sound.

Wishing you an enjoyable and fruitful research.

Approval Date: 27th September 2012

Expiry Date: 26th September 2013

[Signature]

Professor MM Chidzonga