"PLASMA FIBROBLASTIC GROWTH FACTOR 23 LEVELS AS PROGNOSTIC BIOMARKER IN CRITICALLY ILL PATIENTS"

By

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IN

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Under the guidance of

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2020

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Roopashree A

DR ROOPASHREE A

LIST OF ABBREVATIONS

APACHE	Acute Physiology and Chronic Health Evaluation
SAPS	Simplified Acute Physiology Score
FGF	Fibroblast Growth Factor
FGFR	Fibroblast Growth Factor Receptor
CKD	Chronic Kidney Disease
AKI	Acute Kidney Disease
PTH	Parathyroid hormone
ADHR	Autosomal Dominant Hypophosphatemic Rickets
cFGF	Complement Fibroblastic Growth Factor
iFGF	Integrated Fibroblastic Growth Factor
TD	Transmembrane domain
CD	Cytoplasmic domain
TRPC6	Transient Receptor Potential Cation Channel subfamily C, member 6
LVH	Left Ventricular Hypertrophy
HIF-1α	The hypoxia-inducible factor 1-alpha
NF-κB	The nuclear factor kappa-light-chain-enhancer of B-cells
ESRD	End Stage Renal Disease
TNAP	Tissue Non-specific Alkaline Phosphatase
CRP	C-reactive protein
HF	Heart Failure
SHP	Secondary Hyperparathyroidism
ICU	Intensive Care Unit
MPM	Mortality prediction model

ODIN	Organ dysfunction and infection system
SOFA	Sequential organ failure assessment score
MODS	Multiple organs dysfunction score
LOD	Logistic Organ Dysfunction
TRIOS	Three-day Recalibrating ICU outcomes
LOD	Logistic Organ Dysfunction
VALID	Validating Acute Lung Injury biomarkers for Diagnosis
RRT	Renal Replacement Therapy
CPB	Cardio Pulmonary Bypass
VCAM-1	Vascular Cell Adhesion Protein
AGPT2	Angiopoietin 2
ICAM-1	Inter Cellular Adhesion Molecule-1
DAG	Directed Acyclic Graph
AKIN	Acute Kidney Injury Network
CHF	Congestive Heart Failure
ELISA	Enzyme Linked Immunosorbent Assay
CAP	Community Acquired Pneumonia
COPD	Chronic Obstructive Pulmonary Disease
CVA	Cerebro Vascular Accident
DKA	Diabetic Keto Acidosis
GBS	Guillain Bare Syndrome
GI Bleeding	Gastro-Intestinal Bleeding
TBI	Traumatic Brain Injury
OPC	Organo-Phosphorous Compound

LVF	Left Ventricular Failure
DM	Diabetes Mellitus
HTN	Hypertension
IHD	Ischaemic Heart Disease
GCS	Glasgow Coma Scale

ABSTARCT

Background:

It is considered that prognostication is one of the most electrolyte important aspect of critical care. Many parameters and scoring scales have been studied and followed in this aspect. FGF-23 is a recently found FGF and functions as an endocrine hormone that regulates phosphorus homeostasis. It has been linked to predict mortality in CKD and AKI and also recently in all critical patients. So we have undertaken this study to evaluate the role of FGF 23 as a prognostic marker in critically ill patients.

Aims and Objectives:

To evaluate the role of FGF 23 as a prognostic marker in critically ill patients. To study the level of FGF 23 in critically ill patients and to correlate its effects with patient outcome in terms of length of stay in ICU, need for ventilator support, duration of ventilator support.

Results:

In our study, on admission in ICU, mean FGF 23 levels on admission was 332 RU/ml. In survivors it was 312.3 RU/ml(+/- 50.9 RU/ml) and in non-survivors it was 346.8 RU/ml(+/- 109.6 RU/ml) with non-survivors having significant higher levels (p= 0.007). Area under the curve in ROC analysis for APACHE II was more(0.789) when compared to that of FGF 23(0.614) indicating APAPCHE II was a better mortality predictor. There was no difference in duration of mechanical ventilation(p=0.990) and ICU stay(p=0.882) in both the groups and FGF 23 was not a predictor of either of them.

Conclusion:

FGF 23 levels were able to predict mortality(sensitivity 63%, specificity 62%) , but APACHE II was found to be superior to FGF 23 in predicting mortality (sensitivity 70%, specificity 69%) and it can be calculated in any patient using the routinely done investigations and clinical examinations whereas FGF 23 is not cost effective.

KEYWORD: Fibroblast growth factor 23, FGF 23, APACHE II Score.

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INTRODUCTION

Hippocrates considered prognostication as an integral part of medicine.[1] Although research on prognosis has been less when compared to that on etiology and therapy, there has been studies in characterizing the risk factors for death across all areas of medicine.[2] Given the variability among patients and in the etiology, presentation, and treatment of diseases and other health states, a single variable or factor does not often give an adequate information on prognosis. Clinicians use multiple predictors to estimate a patient's prognosis.

Over the course of time, critical care investigators have developed several prognostic systems, including the Acute Physiology and Chronic Health Evaluation (APACHE)[3] and the Simplified Acute Physiology Score (SAPS).[4] These models can consistently and accurately estimate the risk of mortality for populations of critically ill patients throughout the world. But when these models are applied to populations with a particular disease, such as cancer or acute renal failure, among others, their ability to give accurate estimates decreases.[5,6]

The prognostic efficiency of medical parameters, which are scoring systems, estimations and biomarkers, are important for defining critically ill patients. So, to optimize resources and further investigation in this area is warranted.[7]

The family of Fibroblast growth factors (FGF) consists of proteins that regulate cell proliferation, migration, differentiation, and survival [8]. FGF-23 is a recently found FGF and functions as an endocrine hormone that regulates phosphorus homeostasis by binding to FGF receptor (FGFR) and klotho, its coreceptor in the kidney and parathyroid glands. [9-11]

Dysregulated mineral metabolism, like hypocalcemia, hyperparathyroidism, and low circulating levels of 25-hydroxyvitamin D (25D) and 1,25-dihydroxyvitamin D (1,25D), is an important feature of critical illness[12,13] and is more pronounced among patients with Acute Kidney Injury (AKI)[14]. These mineral metabolite abnormalities may be causally associated with adverse outcomes in critical illness through lot of different pathways, including non-

classic effects of vitamin D metabolites on immunity and inflammation[15]. The deranged mineral metabolism is a common complication of chronic kidney disease (CKD) [16], and recently it has been studied in AKI as well[17-20]. Decreased 1,25D levels and hypocalcemia seen with reduced or elevated parathyroid hormone (PTH) levels have been reported in patients with proven AKI.[14,21-23]

David E leaf et al[20] studied the association of FGF-23 with death in critically ill patients with and without AKI and established an independent association. Studies have also indicated FGF 23 levels correlating with outcome in cardiac surgery.[19] In this study we aim to determine whether elevated plasma levels fibroblast growth factor 23 (FGF-23), is prospectively associated with death and morbidity in critically ill patients.

OBJECTIVE OF THE STUDY

Primary objective of the study is to evaluate the role of FGF 23 as a prognostic marker in critically ill patients who need ICU stay of 24 hours or more.

REVIEW OF LITERATURE

Fibroblast growth factor 23 physiology and regulation in critically ill patients

In 1980s, Phosphantonin had been postulated to cause renal phosphate wasting and hypophospatemia based on experiments on hypophosphatemic Hyp-mice. While studying Autosomal Dominant Hypophosphatemic Rickets (ADHR), in early 2000s fibroblast growth factor 23(FGF 23) were discovered as the genetic cause to that renal phosphate wasting disease. [24,25]

FGF-23 Origin and Structure [26]

The fibroblast growth factors (FGFs) belong to a group of proteins involved in embryonic development and metabolic functions [27,28]. All of these derive from the common gene, Fgf13-like, and are comprised of 22 structurally and evolutionarily similar members from Ffg1 to Fgf-23 that conserve a ~120-residue structural domain [29]. Remarkably, Fgf15 and Fgf19 are ortholog proteins in vertebrates, so they are absent in humans and mice, respectively [30].

Phylogenetically, the FGFs family may be divided into seven gene subfamilies that are grouped into three subgroups as per their functions: the intracrine, the paracrine or canonical and the endocrine Fgf genes. The intracrine group includes proteins Fgf11 to Fgf14, which act intracellularly by a pathway independent of the FGF receptor (FGFR) [28]. The canonical subgroup works in an autocrine and paracrine way through the binding and activation of the tyrosine kinase FGFR, which includes heparin/heparan sulfate as a cofactor. This group has five subfamilies: Fgf 1/2/5, Fgf 3/4/6, Fgf 7/10/22, Fgf 8/17/18, and Fgf 9/17/20 [29]. Finally, the endocrine group, with Fgf 19/21/23, acts systemically in a hormonal way through both an FGFR-dependent and FGFR-independent pathway. In contrast to the canonical group, the

endocrine family uses a COOH-terminal domain to activate FGFR, where they are not caught by the extracellular matrix so they can act as circulating factors [27].

The Fgf-23 gene is found on human chromosome 12p3.3, and is comprised of three separate exons and two introns that codify a 32 kDa glycoprotein with 251 amino acids. This full-length protein is recognized as a biologically active hormone, although some studies have suggested that c-terminal fragments may also possess biological activity [31,32]. The COOH-terminal domain (c-terminal; 12 kDa) works as a cofactor by inhibiting iFGF-23 binding to the FGFR/Klotho complex[33]. After the mature protein is released into the circulation, it can be measured as two different isoforms, iFGF-23 (25−FGF-23−251) and the c-terminal FGF-23 (25−FGF-23−179). Commercially available assays quantify circulating FGF-23 levels based on the various epitopes expressed. Assays detecting iFGF-23 recognize 2 epitopes beyond the proteolytic site. By contrast, assays detecting cFGF-23 fragments determine both iFGF-23 and cFGF-23 fragments because of the 2 epitopes captured distal to the cleavage site. The simultaneous recognition of both molecules allows for assessing the production and cleavage of the molecule[33].

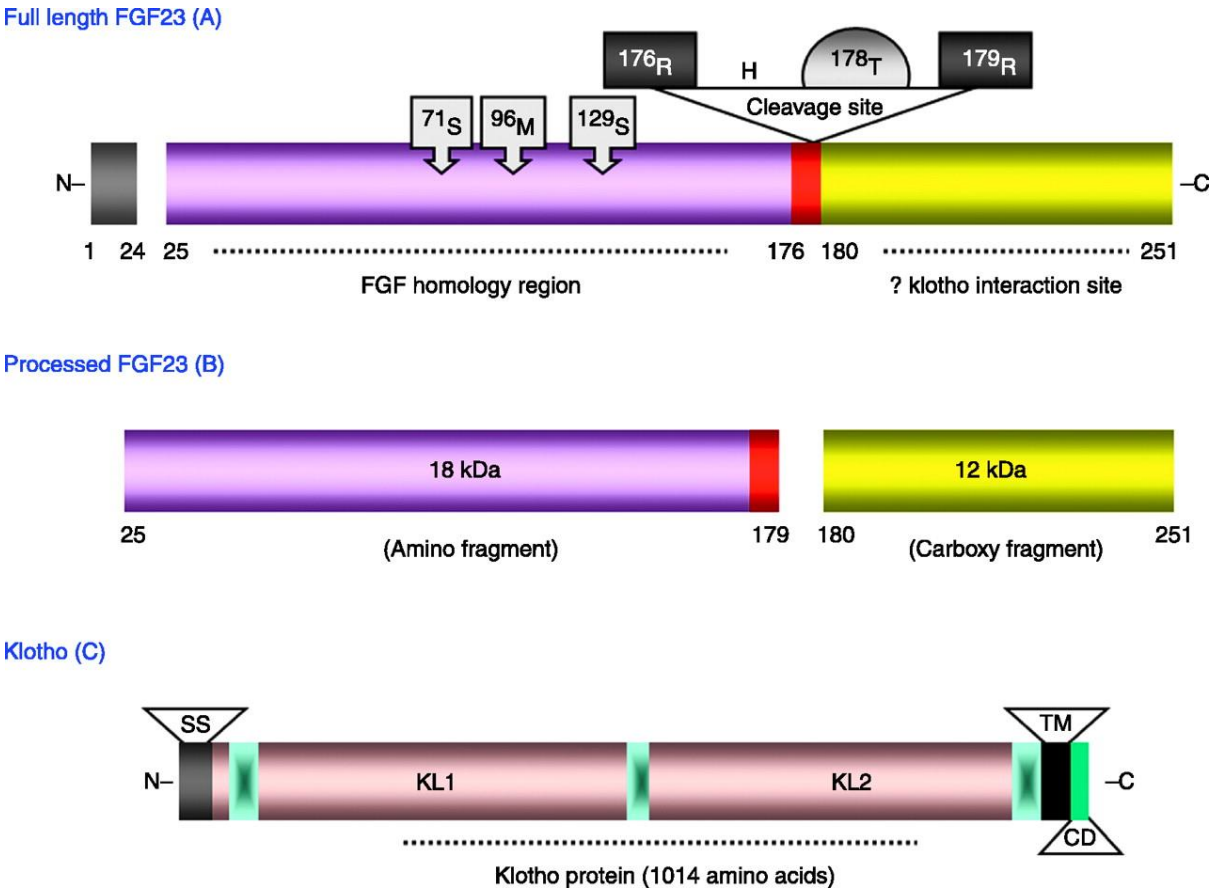


Figure 1 *Schematic diagram*[34] *showing full-length FGF-23 protein (A) with its putative signal peptide (aa 1–24), the amino terminal of FGF-23 (aa 25–179) has the homologous part to other known FGFs; the carboxy terminal of FGF-23 (aa 180–251) might have possible klotho-interacting site. Moreover, aa 176–179 represents the subtilisin-like cleavage site, where full-length FGF-23 protein is cleaved into the smaller 18 kDa amino-terminal and the 12 kDa carboxy-terminal fragments (B). Mutations in 176R and 179R have been reported in patients with ADHR, whereas mutations in 71S, 96M, and 129S have been found in patients affected by FTC. FGF-23 has been shown to be O-glycosylated by the enzyme GALNT3 and the major glycosylation site is believed to be 178T, as seen in the diagram. Klotho protein (C) is composed of 1014 amino acids, and has a putative signal sequence (SS) at its N-terminus and a putative transmembrane domain (TM) with a short cytoplasmic domain (CD) at the C-terminus. The extracellular domain of the klotho protein has two internal repeats (KL1 and KL2) that share sequence homology to the β-glucosidase.*

Mechanisms of Action of FGF-23

The main roles of FGF-23 are to decrease the serum levels of 1,25[OH]2D3 through the inhibition of 1α_hydroxylase and enhance 24_hydroxylase activity[35]. Moreover, it increases phosphaturia by inhibiting phosphate proximal tubular resorption through sodium phosphate cotransporters NaPi2a and NaPi2b [36]. Similarly, PTH also controls renal urinary phosphate excretion by promoting the internalization of NaPi2 cotransporters from the brush border membrane in renal proximal tubules[37]. In early stages of CKD, the increase in PTH is caused in part by a deficiency of 1,25(OH)2D3. The elevation of FGF-23 decreases 1,25[OH]2D3 levels by decreasing renal production and also increasing catabolism. This may explain how in early CKD the elevation in serum PTH is observed once FGF-23 is already increased. FGF-23 tissue-specific functions are dependent on the presence of FGF receptor (FGFR) and in some instances its cofactor αKlotho[38]. Four different FGFRs have been identified, FGFR 1 to 4. Based on the distribution of the different receptors, FGF-23 targets the kidneys, the parathyroid gland, the liver, the heart, the bone, the immune system, and many others [28,29]. Klotho gene encodes a 1014 amino acids type I transmembrane protein with β-glucuronidase activity consisting of two extracellular domains, termed KL1 and KL2 , and is predominantly found on the kidney and the choroid plexus, although it has also been described in the parathyroid gland, the pituitary gland, placenta, skeletal muscle, pancreas, and testis, among others [39-41]. αKlotho was considered must for FGF-23 signalling recognition; however, the discovery of Klotho-independent pathways for FGF-23 transduction has changed after the identification of FGFRs that do not always need αKlotho as a cofactor[42]. αKlotho and FGFRs are also abundantly expressed in the parathyroid glands[43]. Under normal conditions FGF-23 inhibits PTH secretion and production[43,44]; however, in uremia, FGF-23 fails to inhibit PTH release because of down-regulation of the parathyroid FGFR1/Klotho complex[45]. Hence, in ESRD patients on dialysis, FGF-23 levels predict refractory SHP [46].

The molecule αKlotho is important not only because its loss of function is associated with premature aging and target-organ resistance to FGF-23 actions, but also, in ESRD, Klotho depletion is also an early biomarker of disease progression, development of vascular calcifications, and Left Ventricular Hypertrophy (LVH)[47,48]. The full-length αKlotho extracellular domain can be cleaved and released into the circulation as a soluble isoform, namely soluble Klotho (s Klotho), which not only enhances FGFR binding affinity to FGF-23 by 20-fold[49] but also acts as a scaffold to let closer proximity between FGFR and FGF-23, enhancing the stability of the complex[50]. Some studies have suggested that the serum and urine levels of sKlotho may serve as a surrogate marker of renal αKlotho expression[51]. Although strong evidence is still not available, s Klotho could be considered an endocrine mediator targeting various organs without αKlotho expression. In the mouse, sKlotho protects the heart from uremic cardiomyopathy[52] and stress-induced hypertrophy and remodeling; which is achieved by sKlotho-induced inhibition of the transient receptor potential cation channel, subfamily C, member 6 (TRPC6)[53]. Studies show that Klotho deficiency correlates with the development of coronary artery disease, atherosclerosis, myocardial infarction, and left ventricular hypertrophy[54]. Therefore, Klotho might be associated in the regulation of signalling pathways and cell metabolism, being a key factor in cardiac and vascular protection.

Regulation of FGF-23 Production [26]

Regulation of FGF-23 Production

Since the discovery of FGF-23, many have been aiming to evaluate the factors regulating FGF-23 production and cleavage.

1.Vitamin D

The administration of vitamin D rises FGF-23 in both humans and rodents [55]. Also, 1,25[OH]2D3 (calcitriol) increases intestinal absorption of phosphate and calcium, both of which also enhances FGF-23 production. Vitamin D by acting on its specific receptor (VDR) stimulates the promoter region in the FGF-23 gene, which is independent of serum phosphate and calcium. Also, locally produced vitamin D in bones is likely to regulate FGF-23 production [56].

2. Phosphate

Phosphate sensing receptors are not yet known[57], but it is proven that phosphate load, even without high serum phosphate, stimulates FGF-23 production. In CKD patients and patients on dialysis, the high serum phosphate levels are associated with elevated FGF-23 levels [58].

3. Calcium and PTH

PTH facilitates the transcription of FGF-23 in a calcitriol-independent way [59]. The reports that PTH activates the orphan nuclear receptor Nurr1 has shown the mechanism by which PTH increases FGF-23. In dialysis patients, FGF-23 correlates directly with serum levels of phosphate and PTH, and inversely with the serum calcium concentration [60].

It is likely that hypocalcemia decreases circulating FGF-23 to prevent a decrease in calcitriol, which would worsen a situation of calcium deficiency[61]. VDR null mice fed a high-calcium diet had increased circulating FGF-23, which suggests that the regulation of FGF3 by calcium is not dependent on VDR. In the general population and CKD patients, intake of an enriched calcium diet is associated with increased FGF-23. In dialysis patients, the calcium effect on

FGF-23 production is more evident if phosphate is within the normal range. A decrease in calcium below 8 mg/dL reduces the elevation of FGF-23 induced by high phosphate. The reduction of PTH after the administration of calcimimetics is seen with a decrease in FGF-23, which is likely related to the concomitant decrease in both serum calcium and phosphate[62].

4. Inflammation and Iron Deficiency

There may be bi-directional interaction between inflammation and FGF-23 production[63]. Since both inflammation and elevation of FGF-23 are associated with mortality, the understanding of this loop is of high importance in clinical practice. Either acute or chronic inflammation may increase FGF-23 transcription and cleavage [64]. The induction of acute inflammation in wild-type mice not only decreased the serum iron and increased serum ferritin levels but also resulted in an elevation in bone Fgf-23 mRNA expression that was accompanied by a rise in cFGF-23, while iFGF-23 remained unchanged. Whereas, the induction of chronic inflammation in a murine CKD model was followed by a concurrent elevation in both iFGF-23 and cFGF-23, although the increase in c-terminal fragments was greater than that of iFGF-23[64]. Inflammation and iron deficiency enhance the activity of the hypoxia-inducible factor 1-alpha (HIF-1α) signalling, thereby increasing Fgf-23 transcription. Therefore, inflammation and iron deficiency facilitate not only FGF-23 transcription but also cleavage. In healthy women with iron deficiency, the administration of intravenous iron reduced cFGF-23, whereas iFGF-23 increases transiently, may be due to the reduction of FGF-23 cleavage [65].

In CKD and dialysis patients, the high serum phosphate correlates with high levels of FGF-23, which in turn is seen with an elevation of C-reactive protein[66]. Inflammation may not only modulate FGF-23 production but also cleavage since the relative effect of inflammation on the

increase of cFGF-23 is 3 times higher than that of iFGF-23. A mechanism whereby inflammation may enhance FGF-23 production is the activation of the nuclear factor kappa-light-chain-enhancer of B-cells (NF-κB) pathway, which has been linked to FGF-23 transcription. However, NF-κB may also increase FGF-23 production through the upregulation of HIF-1α, which is known to raise FGF-23.

5. Erythropoietin

A few studies have shown an association between FGF-23 and erythropoiesis[67]. Although increased FGF-23 reduces bone marrow EPO expression, EPO enhances FGF-23 transcription; the exact mechanism remains uncertain, although it is likely to be independent of iron and Klotho. The administration of EPO to patients with AKI was followed by an elevation in circulating FGF-23. These results are in congruence with other additional studies in kidney transplant recipients and dialysis patients[68].

6. Others

a. Adiponectin

The adipose tissue releases many factors with endocrine functions. Indeed, adiponectin limits renal damage and accelerates renal recovery after kidney injury in animal models[69]. Chronic kidney disease upregulates adiponectin expression. Furthermore, adiponectin increases the renal excretion of calcium and thereby the risk of osteoporosis. Recently, adiponectin has been shown to couple with systemic mineral homeostasis and renal handling of phosphate and calcium. High adiponectin decreases kidney secretion of αKlotho and FGF-23 production by

osteocytes, although it enhances the renal loss of calcium. This is mediated by the activation of renal ADIPOR1 and ADIPOR2 receptors[70].

b. Insulin

Diabetes is associated with raised FGF-23[71]. Also, raised cFGF-23 has been associated with insulin resistance, obesity, resistin, and HOMA-IR. These correlations may be mediated through inflammation since these patients also had higher levels of IL-6, CRP, and IL-10. A recent report has delineated insulin-dependent signalling for FGF-23 synthesis. Insulin and insulin-like growth factor (IGF-1) decrease FGF-23 production by activating the PI3K/PKB/Akt/FOXO 1 signaling[72].

c. Aldosterone

The discovery of the interaction between FGF-23 and aldosterone regulation has been a significant one. By activating the FGFR1/α-Klotho/ERK pathway, FGF-23 increases sodium reabsorption through sodium/chloride cotransporter in distal tubules with a significant impact on fluid overload and an elevation of blood pressure[73]. In parallel, aldosterone enhances FGF-23 secretion, which in turn increases the expression of angiotensin II in cardiac myocytes. In dialysis patients, volume overload is seen with elevated FGF-23[74].

d. Regulation of FGF-23 Production by Bone Cell Factors

Production of FGF-23 is under regulation at the bone level by the gene with homology to endopeptidases situated in the X chromosome (PHEX) and the dentin matrix protein 1 (DMP1). PHEX is a 106 kDa protein expressed by osteoblast and osteocytes. Inactivating the mutation

of PHEX leads to excessive Fgf-23 gene transcription. Whereas, overactivation of PHEX decreases FGF-23. It is likely that the cleavage of the intact protein is the main regulating function of PHEX[75]. The DMP1 is a 94 kDa protein expressed in osteoblasts and osteocytes that is critical in bone mineralization. Even though its inhibition seems like PHEX inactivation, its overactivation does not lead to FGF-23 elevation.

FGF-23 in the Kidney- actions[76]

FGF 23 is known to inhibit control of renal 1α_hydroxylase (CYP27B1) transcription, which is main factor for production of 1,25(OH)2D3, as shown in knock-out mice preliminaries. This is its most important function rather than the phosphaturic work. Likewise, without the ligand FGF-23 or of its co-receptor αKlotho, the meticulous endocrine regulation of 1α_hydroxylase transcription fails, causing improperly high production and effect of this enzyme. The impact of 1α_hydroxylase overexpression is increased 1,25[OH]2D3 levels, causing hypercalcemia, hyperphosphatemia, ectopic calcifications, weakened bone mineralization, and early death in mice without αKlotho and Fgf-23. The foremost function of 1,25[OH]2D3 in mineral metabolism is the enhancement of absorption of calcium and phosphorus in the gut. The significant function of 1,25[OH]2D3 overproduction in mediating the phenotype of αKlotho− and Fgf-23− mice is underscored by the removal of vitamin D signalling pathway resulting in saving the Fgf-23− and αKlotho mice. In human subjects with loss-of-function mutations in FGF-23 or αKlotho there was increased vitamin D levels and soft tissue calcifications[77].

Intracellularly FGF-23 suppresses 1α_hydroxylase transcription. Though the importance of this is known, the exact mechanics are yet unclear. It appears to have a complex intracellular signalling pathway. 1α_hydroxylase is dominant in proximal convoluted tubules (PCT). PCT

and distal convoluted tubules (DCT) bear the coreceptor α-Klotho and also FGFR-1, 3, and 4, but insignificant FGFR- 2[78]. All FGFRs are functionally receptor tyrosine kinases. They start the intracellular phosphorylation pathway after ligand mediated dimerization. FGF-23-induced suppression of 1,25[OH]2D3 production was absent in mice in which FGFR-1 was targeted and deleted. So, it can be concluded that FGFR-1c could be the FGFR seeing to the suppressive effects of FGF-23 on renal tubular 1,25[OH]2D3 production. FGFR3 and 4 may also be involved, but it appears to be of minimal effect. Genetic ablation of Fgfr3 and Fgfr4 will see to it the renal 1α_hydroxylase expression in hyp-mice, and there is more of endogenous-FGF-23[79]. FGF-23 mediated suppression of 1α_hydroxylase transcription works under extracellular signal-regulated kinase-1 and -2 (ERK-1/2) stimulation, though little is known about the ERK-1/2 mechanism. The FGF-23-mediates the transcriptional activity of the 1α_hydroxylase gene. This process is under the regulatory elements situated in introns of the Mettl-21b gene. FGF-23 regulates the 1α_hydroxylase which is not dependent on 1,25[OH]2D3 and vitamin D receptor (VDR)[80].

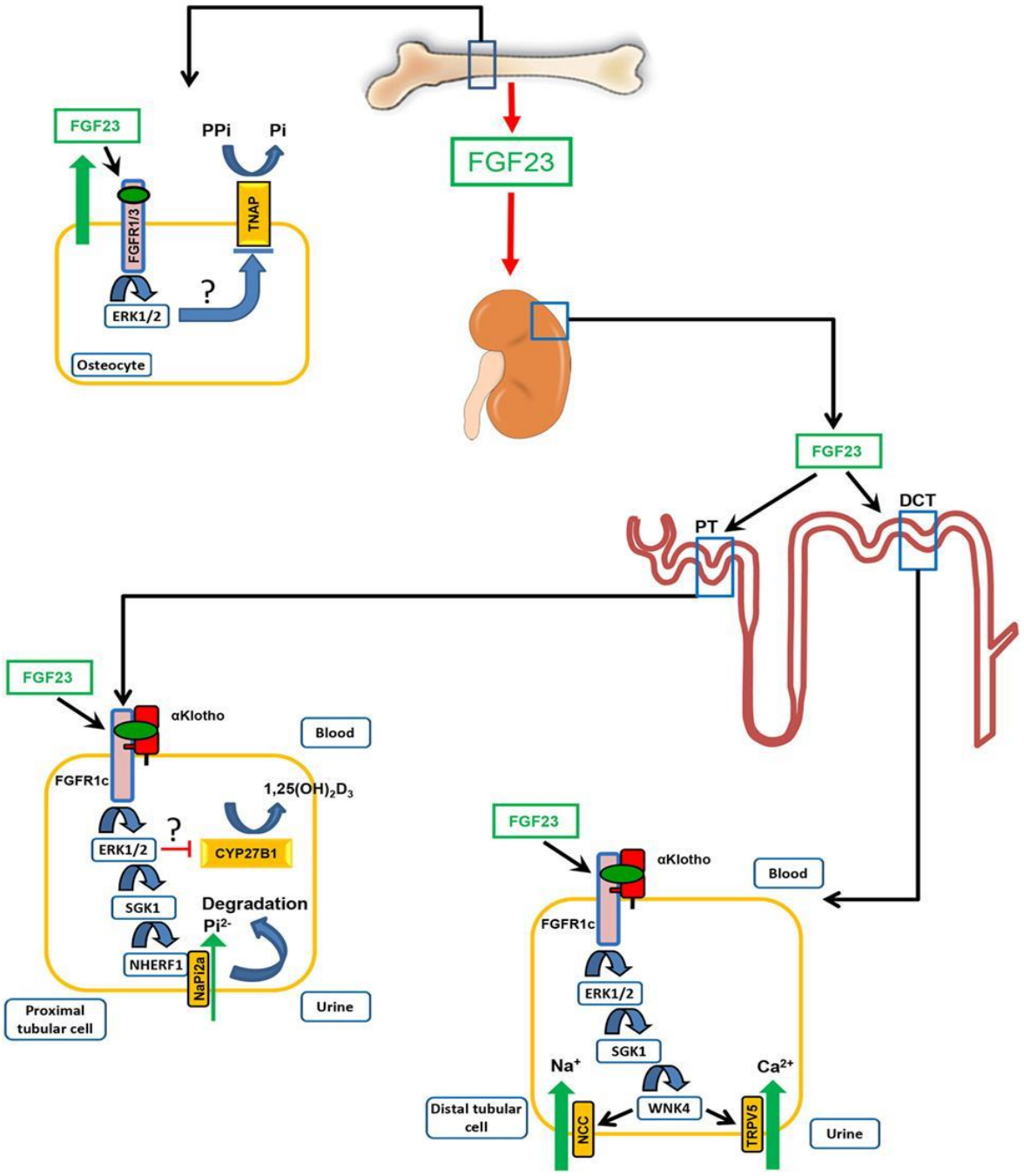


Figure2. *Physiological roles of fibroblast growth factor-23 (FGF-23)*[76]*. Bone cells, osteoblasts, and osteocytes are the ones producing FGF-23. Kidney. FGF-23 acts on PCT and DCT. αKlotho is mandatory for FGF-23 to bind to FGF receptor-1c (FGFR-1c). The rate-limiting enzyme for vitamin D hormone (1α,25[OH]2D3) production is 1α_hydroxylase (CYP27-B1). FGF-23 inhibits this enzyme and also the reuptake of phosphate in PCT. ERK-1/2 is required for this deactivation. Though the exact mechanics of this signals are not yet understood. ERK-1/2 and serum/glucocorticoid-regulated kinase-1 (SGK-1). These carries put the inhibition of phosphate reabsorption in PCT by FGF-23. This phosphorylates the scaffolding protein Na+\H+ exchange regulatory cofactor [NHERF]-1. All this trigger internalizing and degrading of the sodium phosphate cotransporter NaPi-2a. This leads to less NaPi-2a in apical membrane. So, this is not there for phosphate reuptake from urine. In DCT,*

FGF-23 enhances reabsorption of Ca^{2+} and Na^{+} by promoting the sodium-chloride cotransporter NCC by a signal cascade involving ERK-1/2, SGK-1. FGF-23 from osteocytes inhibits bone mineralization by downsizing
tissue nonspecific alkaline phosphatase (TNAP) transcription in a α-Klotho non-dependent manner by FGFR-1 / FGFR-3 driven stimulation of ERK- 1/2. TNAP is required for regular mineralization of bone. This is achieved by breaking the inhibitor pyrophosphate.

Parathyroid hormone and FGF-23 regulate 1α- and 24_hydroxylase (CYP24-A1) expression inversely. FGF-23 suppresses 1α_hydroxylase, but induces the expression of 24_hydroxylase. PTH effects oppositely. 1α-hydroxylation is activation of the 25-hydroxy vitamin D into the functionally active substance, however 24-hydroxylation is an inactivation process[81]. 1,25[OH]2D is a strong inducer of 24_hydroxylase which stimulates self-degradation. 24_hydroxylase transcription can have FGF-23 as a direct modulator, though not well established. Fgf-23 negative mice and also in wild type mice treated with rcFGF-23 were studied. Suggestions were made that FGF-23 signal may directly stimulate 24_hydroxylase. Studies in VDR knock-out mice indicate that the FGF-23 regulation of 24_hydroxylase is not a direct mechanism, but is depending on VDR. FGF-23 regulated induction and the PTH regulated suppression of 24_hydroxylase is not present in 1α_hydroxylase knock-out mice. All this suggest that FGF-23 and PTH regulation of 24_hydroxylase expression is is not direct through altered 1,25[OH]-2D3 production and subsequent changes in promoter function of 24_hydroxylase.

Inhibition of cellular phosphate reuptake from urine in PCT leads to phosphate loss under the influence of FGF-23. The phosphaturic action of FGF-23 is functionally less vital whereas the 1α_hydroxylase suppression is more important. The phosphaturic and the 1,25[OH]-2D3 decreasing action of FGF-23 ensure that hyperphosphatemia does not occur: these are achieved by increasing renal elimination of phosphate and reducing the absorption of phosphate in intestine respectively. 1,25[OH]-2D3 and phosphate stimulate FGF-23 in bone, so the

phosphaturic and 1,25(OH)2D3 decreasing actions of FGF-23 end up creating a negative feedback between bone and kidney which is looping.

FGF-23 is known to be a regulator of both vitamin-D and phosphate metabolism. It directly mediates the handling of calcium-sodium in the DCT portions of nephrons in the renal system. Mice with skeletally mature Fgf-23/VDR and Klotho/VDR compound mutation were found to be having renal calcium wasting, renal salt wasting and subsequent hyponatremia, hypovolemia, and hypotension. The most important aspect of FGFR1c/Klotho complex seems to be its pivotal role of receptor complex in the DCT. DCT-specific deletion of Fgfr1 recapitulates the renal calcium wasting as noticed in Fgf-23/VDR compound mutant mice has been found to be reason for the same. Fgf-23 and Klotho deficient mice show a down-regulation of DCT - TRPV5 and NCC membrane expression, there by resulting in renal calcium and sodium wasting. This has been observed despite counter-regulatory measures increasing circulating PTH and aldosterone are functional. thus, it can infer that the calcium- and sodium-conserving functions of FGF-23 in DCT are of prime physiological importance. The enhanced renal conservation of calcium assists in maintaining blood calcium levels despite the suppression of 1,25[OH]2D3 synthesis brought upon by up-regulated FGF-23 secretion.

PTH and FGF-23 have similar roles in PCTs and DCTs. These hormones inhibit phosphate reabsorption in PCTs by achieving NHERF-1 phosphorylation, and thereby enhancing calcium re-absorption in DCTs by targeting focal expression of TRPV5. Albeit the signal mechanisms might be different, the proximal and distal renal target molecules of PTH and FGF-23 are essentially same. Also, it has been observed that the absence of FGF-23 signalling in Fgf-23 deficient mice would result in partial renal resistance to the phosphaturic and calcium-conserving functions of PTH [82]. Likewise, the reduction in PTH signalling in human subjects with hypoparathyroidism has been suggestive of partial resistance to the phosphaturic actions of FGF-23. Hence, it has been postulated that there is complex interplay among these

hormones. The vital physiological role of FGF-23 also is to enable normal responsiveness to PTH signalling in the kidney and also in bone[82].

FGF-23 in Bone-Functions

Fibroblast growth factor-23 has also been postulated to have physiological role in bone mineralization and haematopoiesis. FGF-23 has been noted to have a strong suppressor activity of transcription of tissue non-specific alkaline phosphatase (TNAP) mRNA in bone cells in a Klotho-independent manner[83]. TNAP has been found to be a necessary for the regulation of bone mineralization, where in it acts via cleaving the mineralization inhibitor pyrophosphate which is secreted by osteoblasts to prevent early mineralization of osteoid. Shalhoub et al have reported that FGF-23 suppresses TNAP expression in mouse osteoblast-like cells in a FGFR1-dependent pathway.[84] This effect may be further enhanced by soluble Klotho. So, further research is implicated to elaborate the FGF-23 mediated suppression of TNAP in bone cells whether it is mainly facilitated through FGFR-1, FGFR-3, or both. The expression of Klotho in bone is very low[85]. So, it is unlikely that Klotho expression in bone cells is enough to enhance FGF-23 binding to FGFRs in osteoblasts and osteocytes. Nevertheless, it can be assumed that because of the local production of FGF-23 in osteocytes, the concentration of FGF23 within the canalicular system is sufficiently high for auto-/paracrine, Klotho-independent signalling through FGFRs in bone. Therefore, locally produced FGF-23, not only could contribute to impaired mineralization under the conditions of profound bony FGF-23 secretion such as in Hyp-mice[86], but could also serve as a physiological inhibitor of bone mineralization achieved by down-regulating TNAP expression. Similarly, it is found that an up-regulation of Tnap mRNA in Fgf-23 lacking, Fgf-23\VDR compound mutant mice compared with wild-type and VDR control mice[83]. Anyhow, the relevance of this mechanism

in the context of physiological ranges of FGF-23 secretion remains unleaded and calls for future research.

Fgf-23 void mice show increased erythropoiesis[87]. Erythropoiesis has been suppressed by the injection of the recombinant FGF-23 in unaltered mice. In these mice population, it has been found that the suppression of erythropoiesis by inhibition of FGF-23 signalling. So FGF23 could be a physiological regulator of erythroid lineage commitment in the bone microenvironment. However, the signal-mechanisms causing this effect are unknown, and future studies are implicated to establish the relevance of this effect in relation to the physiological regulation of erythropoietin mediated erythropoiesis.

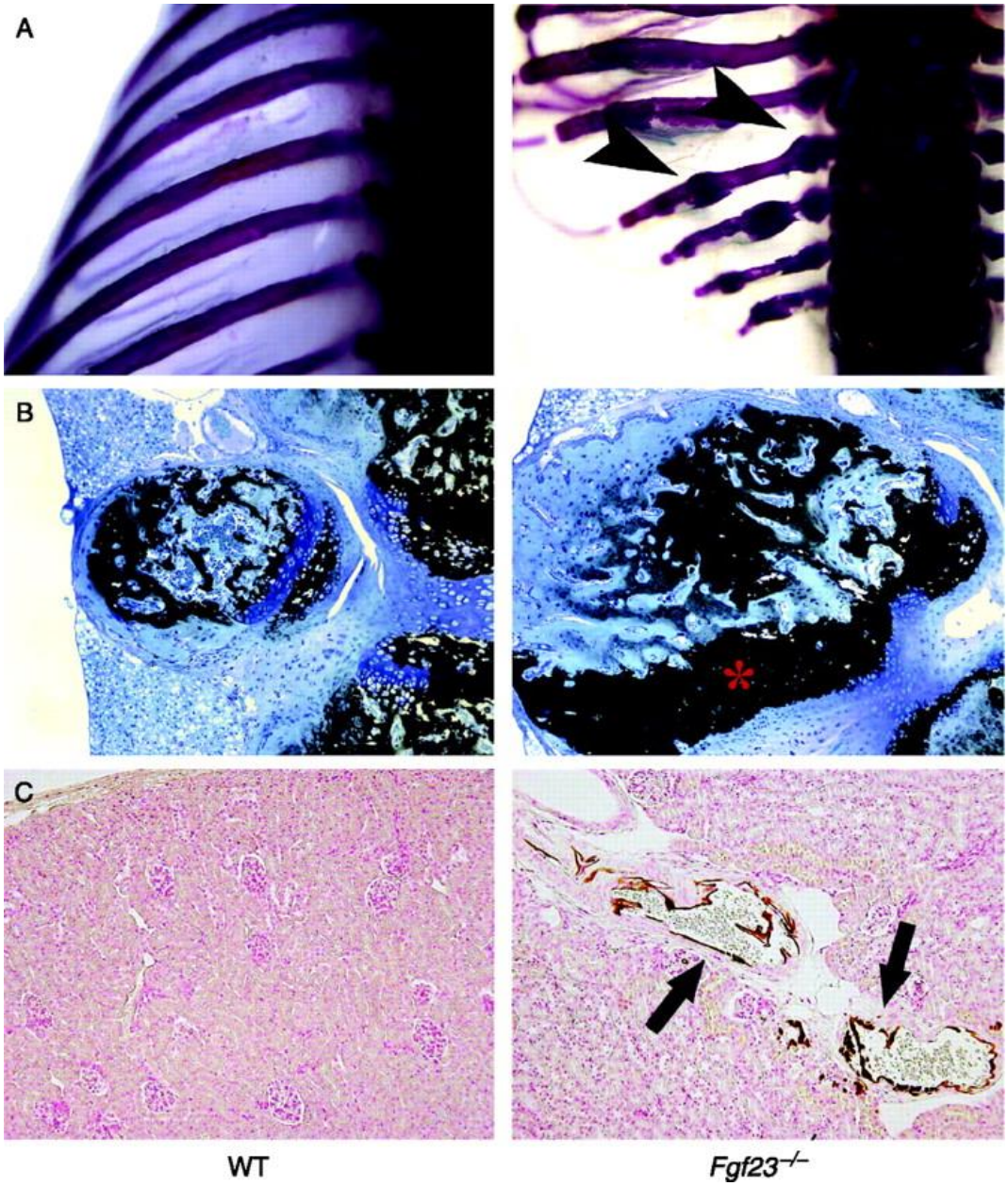


Figure 3 Deranged mineralization in bone and kidney of Fgf-23 knock-out (Fgf-23−/−) mice.[34] *Abnormal nodular lesions (depicted by arrows) in the ribs of Fgf-23−/− mice, compared with control (A). Histomorphometric analysis shows an increased mineral deposition (black) in the mutant ribs (depicted by red asterisk- B). In Fgf-23−/− mice, skeletal mineralization shows heterogeneous distribution, where increased mineral deposition is also accompanied with unmineralized osteoid (light blue) generation (B). Extensive soft tissue mineralization is a specific feature in Fgf-23−/− mice, as depicted here in the kidney (C).*

Physiological Functions of FGF-23 in The Heart

Left ventricular hypertrophy (LVH) is often seen in patients with advanced CKD[88]. FGF-23 has also been associated with an adverse effect on the heart. This has to be a Klotho-independent manner as the heart lacks αKlotho expression[89]. Therefore, FGF-23 targets the heart directly by acting on FGFR4 with subsequent activation of the PLCγ/calcineurin/NFAT signalling pathway, which leads to cardiac hypertrophy, fibrosis, and heart failure. This has been established in in vitro studies and non-CKD mice models. Inhibitors for FGFR4 stop such a detrimental effect. As described above, the FGF-23 increase leads to reductions in Klotho. Reductions in sKlotho in itself also leads to cardiac injury in animal models. Also, the use of recombinant sKlotho in a CKD model of Klotho-deficient mice decreased cardiac remodelling irrespective of the existing FGF-23 levels[89]. LVH is likely because of the Klotho deficiency rather than because of FGF-23 excess since even with normalization of both phosphate and FGF-23, LHV was still seen in Klotho-replete cultures of cardiac myocytes [89].

FGF-23 could also aggravate blood pressure, inflammation, and CKD progression[90], all of which are seen as LVH develops. FGF-23 is such a strong cardiac remodelling factor that it effects cardiac cells irrespective of other pro-hypertrophic factors. The cardiac expression of FGF-23 and FGFR4 is increased in human cardiomyocytes [91]. In these subjects, cardiac FGF-23 correlate with cross-sectional area of cardiac myocytes and with brain natriuretic peptide (BNP). sKlotho has been identified in lysates of human myocardial tissue, however αKlotho is not present in cardiac myocytes, [91]. However, sKlotho was decreased in patients with LVH and its levels correlated inversely with ESRD duration and the type of renal replacement therapy. Noticeably, recent studies have established that the duration of the exposure to an increment in FGF-23 is more vital for cardiac remodelling than the magnitude of FGF-23 elevation[92].

FGF-23 upregulates the expression of TGF-β in cardiac fibroblasts, which initiates the cardiac fibrosis through the β-catenin pathway. FGF-23 also triggers the proliferation of mice cardiac fibroblasts and collagen I and II synthesis, especially after myocardial infarction[93]. In myocardial isolates from dialysis patients, cardiac fibrosis correlated directly with the dialysis vintage and inversely with the significant deficiency of sKlotho in cardiac myocytes. This might be prevented by the administration of recombinant sKlotho as it inhibits the transient receptor potential cation channel, subfamily C, member 6 (TRPC6) in the mouse cardia.

All the effects of FGF-23 on the heart need not be that harmful; FGF-23 may increase intracellular calcium in cardiac myocytes, increasing contractility, still, this may lead to a greater risk of arrhythmia[94].

Physiological Functions of FGF-23 in Liver

The liver has one of the highest expressions of FGFR4[95]. As with the heart, FGF-23 targets the liver by activating the FGFR4/PLCγ/calcineurin/NFAT pathway. This signalling pathway causes FGF-23 to trigger the production of inflammatory cytokines such as C-reactive protein (CRP), IL-6, IL-12, and TNFα. The inflammatory cytokines producing cells of liver, Kupffer cells, respond to FGF-23 triggering[96]. Also, FGF-23 may promote hepatocyte proliferation and did not seem to produce hepatic tissue injury as there was no elevation in liver enzymes. May be due to the correlation with insulin resistance and diabetes, FGF-23 and reduced circulating calcifediol were independently associated with non-alcoholic fatty liver disease as reported in a study.[97]

Patients with end-stage renal disease (ESRD) show inflammation due to several reasons, including the dialysis itself[98]. Elevated FGF-23 trigger cytokine production, which then leads to systemic inflammation. Similar to the heart, FGFR4 could be pharmacologically targeted by the use of FGFR4 inhibitors. More studies are necessary to formulate appropriate strategies to reduce FGF-23-mediated cytokine production.

Physiological Functions of FGF-23 in Immune System

Infections are often seen in patients with CKD than in the general population. In studies FGF-23 has been corelated strongly in infections in ESRD patients[99]. As it prevents β2-integrin activation FGF-23 inhibits the triggering, binding, and migration of neutrophils. This action seems to be Klotho-independent and is mediated by FGFR2, although FGFR1 and FGFR4 are both expressed in neutrophils. In addition, it has been established that FGF-23 reduces the expression of CD11b integrin, thereby decreasing neutrophil chemotaxis. The function of recombinant FGF-23 on neutrophils in rodent models is dose-dependent and is mostly observed with high levels of FGF-23, as elevated as those seen in dialysis patients [99]. Additionally, FGF-23 upregulates TNFα production and downregulates calcitriol production in monocytes.

Physiological Functions of FGF-23 in Other **Organs**

Other effects of FGF-23 have been studied and established in organs such as the brain, lungs, skeletal muscle, and endothelium, but the mechanisms of action have not been understood.

Various types of brain cells possess complex FGFR1-αKlotho, but their roles remain uncertain. An elevation in FGF-23 levels is seen with reduced neuronal ramifications and increased synaptic density. This is carried out by the activation of PLCγ signalling and happens in the absence of αKlotho[100]. However, a high phosphate intake could reverse this effect, this contradicts the hypothesis of a potentially detrimental role of FGF-23 on memory cells.

CKD patients who are on dialysis frequently complain of muscle weakness. In fact, sarcolemma shortening is noticed in ESRD patients. In animal studies, FGF-23 reduces muscle strength, an effect that should be mediated by FGFR4 since, like the cardiac myocyte, skeletal

muscle lacks the expression of Klotho. Studies on the effects of FGF-23 on skeletal muscle are not uniform since other works indicate that high FGF-23 may enhance exercise performance[101]. Even though the lung tissue expresses FGFR1, FGFR2, FGFR3, and FGFR4, there is not enough strong evidence indicating whether FGF-23 has specific effects on the lungs. It could be that FGF-23 increases IL-8 secretion by epithelial cells, though there is no evidence of a detrimental effect. Reports points to a lack of Klotho as a risk factor for lung emphysema, but the evidence is less and needs further study.

S Klotho expression protects the endothelium from uremic aging[102]. Though, results derived from studies investigating the effect of FGF-23/Klotho on the endothelium are conflicting. However, some studies have linked FGF-23 with endothelial dysfunction, the studies in CKD patients are conflicting[103].

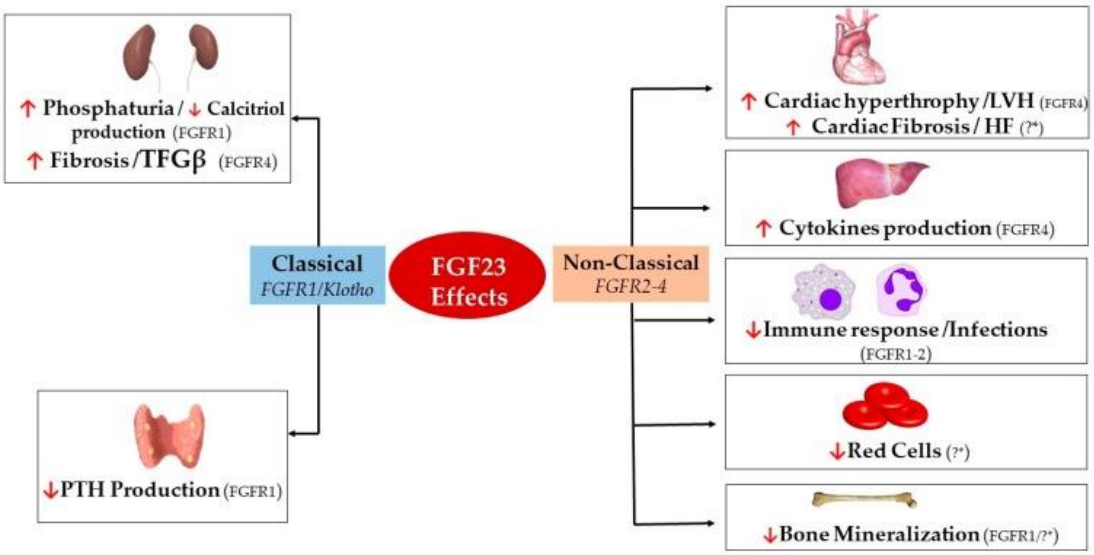


*Figure 4 Summary of classical and non-classical clinical effects of FGF-23 on various organs.[26] TFGβ, transforming growth factor βeta; PTH, parathyroid hormone; LVF, left ventricle hypertrophy; HF, heart failure. ↓ Decrease; ↑ Increase; * Presumably Klotho-independent.*

Prognostic scoring in critical care unit[104]

In early days, predicting outcome in critical illness was mainly on the subjective judgment of the doctors. The ever-developing intensive care units (ICUs) had the need for quantitative and clinically relevant surrogate outcome systems in order to evaluate the effectiveness of treatment regimes. So, scores have been developed and used for this purpose. The outcome in an ICU patient depends on several factors from day 1 in the ICU and from there on the patient's further course in ICU. For this purpose, many scoring models have been developed. Most of these models are known simply by their acronym. A scoring model usually consists of two parts – a score (a number assigned to disease severity) and a probability system (equation predicting the probability of hospital death of the patients). A system refines the power of scores or scales to be useful in comparing different groups of patients for the purpose of treatment, triage or comparative analysis and thereby helping in various aspects of prognostication and decision making. They also help in obtaining an increased understanding of the efficiency of treatment and optimizing the use of hospital resources. Hence these aid in the formulation of treatment standards. An ideal scoring system should possess a high predictive power starting from day one, it should not be constrained to certain cut-off-points and should be calculated according to the well-established systems used for such a purpose with specific β-coefficients. To transform the severity index into a probability of mortality in the hospital a logistic regression equation is used. The ideal system would have to be well-validated, calibrated and discriminated. Validity is usually used to evaluate the performance of the prediction system by testing in the dataset that was used for system development. Calibration assesses the accuracy of the degree of corresponding consistency between the calculated probabilities of death produced by a system and the actual death experienced by patient population. Calibration can be statistically worked out using formal goodness of fit

tests. Discrimination is efficiency of the model to separate patients who expire from patients who live on, based on the estimated probabilities of death. Discrimination is expressed in terms of sensitivity, specificity, false positive rate, false negative rate, positive predictive value, misclassification rate, area under the receiver operating characteristic curve and concordance.

Types of ICU Scoring Systems[104]

In most of the scoring models, scores are derived from data collected on the day one of ICU by:

- Acute physiology and chronic health evaluation (APACHE)
- Simplified acute physiology score (SAPS) and
- Mortality prediction model (MPM).

Few other scores are repetitive and collect data each day throughout the ICU stay or for the initial 3 days –

- Organ dysfunction and infection system (ODIN),
- Sequential organ failure assessment (SOFA),
- Multiple organs dysfunction score (MODS),
- Logistic organ dysfunction (LOD) model and
- Three-day recalibrating ICU outcomes (TRIOS).

Scores can be subjective or objective. Subjective scores are given by a panel of experts who select the variables and assign a value to each variable based on their individual opinion. Examples of this are APACHE II, ODIN and SOFA. Objective score variables are collected utilizing the logistic regression modelling techniques and clinical judgment to get ranges and to assign weights. APACHE III, SAPS II, MPM II, MODS, LOD score (LODS) and TRIOS are few of objective scores.

Many reports have shown the efficiency of scoring models in predicting hospital deaths and most of the available models are comparable in terms of outcome prediction.[105,106] Prediction systems should however, periodically be updated to accommodate the changes in medical practice and case prevalence over time. In their study Meyer et al.[107] demonstrated that among patients who were predicted by clinical judgment and APACHE II score to die, more than 40% actually lived. They also inferred that no model is reliable for predicting the death of surgical ICU patients.

APACHE II[3]

APACHE II was developed in 1985 using a database of North American intensive care patients, and it is the severity of disease classification model. It takes from a point score based upon values of 12 routine physiologic values (taken on the day of admission), age of the patient and previous health condition to provide a general estimation of severity of disease. An integer score from 0 to 71 is then calculated based on these values; higher scores predict a more severe disease and a higher risk of death. APACHE II can prognostically categorise acutely sick patients and assist clinicians comparing the efficiency of new or differing methods of therapy. If a variable is not measured, it is given zero points. Hospital death is predicted using the APACHE II score, the principal diagnostic category with which the patient is admitted to ICU and also depending on if the patient underwent emergency surgery.[3] The estimated risk of hospital mortality is determined using logistic regression equation, using specific beta co-efficient derived for this purpose. The APACHE II system is neither very sensitive nor specific in terms of death prediction. The major drawback of this scoring model is that many patients have several co-morbid preconditions and choosing only one principal diagnostic category can become very difficult. Also, the physiological values are all dynamic and can be influenced by multiple factors, like ongoing resuscitation and management, so a time bias is present. This is

an important consideration when managing patients in the ICU especially with currently increased emphasis on the significance of an early goal directed therapies. There is a possibility of overestimation of predicted mortality because of all these factors.

SAPS II

In 1993 by Le Gall et al.[108] used SAPS II as measure of the severity in ICU patients. The score includes 17 variables: 12 physiologic factors, age, type of admission and three disease-related factors.

Parameters in SAPS II

- Age,
- Heart Rate
- Systolic Blood Pressure
- Temperature
- Glasgow Coma Scale
- Mechanical Ventilation or CPAP
- PaO2
- FiO2
- Urine Output
- Blood Urea Nitrogen
- Sodium
- Potassium
- Bicarbonate
- Bilirubin
- White Blood Cell
- Chronic diseases

- Type of admission

Similar to other models, the SAPS II score takes the least value of selected variables, on the day of admission. The score can vary between 0 and 163 points.

- 0-116 points for physiological variables,
- 0-17 points given to age and
- 0-30 points for previous comorbidities.

Probability of mortality is then estimated with logistic regression. The discrimination and particularly the calibration of the SAPS II model do not apply when fitting into to a new population.

MODS

Marshall et al.[109] developed an objective scale in 1995 to estimate the severity of multiple organ dysfunction as an outcome in ICU and put these into test in 692 patients. MODS consists a score based on six organ dysfunctions. Respiratory with oxygenation, Renal with serum creatinine Cardiovascular with pressure adjusted heart rate, Hematologic with platelet count and Neurologic with Glasgow Coma Score. Scores range from 0 to 4, to a maximum of 24. Hospital death is then estimated based on the total scores. This score associated in a graded fashion with the ICU death rate, when applied on day one of ICU admission as a prognostic indicator and also when applied over the ICU stay as an outcome indicator. The score presented good discrimination and that mortality depends not only on the admission score but also on the course of ICU stay. Therefore, MODS could be useful as an alternative end point for clinical studies in ICU patients.

Sequential Organ Failure Assessment

European Society of Intensive Care Medicine in 1994 gave a Sequential organ failure assessment score which was further revised in 1996.[110] SOFA subjective scoring was evaluated by Vincent et al.[111] in 1998. SOFA was intended to quantify the severity of critical illness which was based on the organ dysfunction data of six organ failures. SOFA is scored on a scale of 0-4. Further studies have considered the maximum score plus the maximum change during stay and established that the latter has an inferior prognostic value than the former. The general course of the patient's status during the entire ICU stay is also considered. Even though SOFA score cannot be directly converted to mortality, a rough estimate of death risk may be made.[110-112]

Table 1: Sequential Organ Failure Assessment (SOFA) Score[110]

Variable Points	0	1	2	3	4
Pao_2 /Fio_2(mmhg)		<400	<300	<200	<100
Platelet Count(×103µL)		<150	<100	<50	<20
Glasgow Coma Scale		13-14	10-12	6-9	<6
Bilirubin(mg/dl)		1.2-1.9	2-5.9	6-11	>12
MAP	No hypotension	MAP Below 70	On Vasopressors, Dopamine<5µg/kg/min or Dobutamine (any dose)	Dopamine>5µg/kg/min or Epi/Norepi<0.1µg/kg/min	Dopamine >15µg/kg/min or Epi/Norepiµg/kg/min
Creatinine(mg/dl)	<1.2	1.2-1.9	2.0-3.4	3.5-4.9	5.0

Review of FGF-23 as prognostic marker in critical care.

David E leaf et al[23] in 2013 evaluated the correlation between vitamin D metabolites and clinical outcomes in patients with AKI. In a total of 30 participants with AKI and 30 controls from general wards and intensive care units at a tertiary care hospital, they measured plasma levels of 25-hydroxyvitamin D [25(OH)D], 1,25(OH)2D, 24R,25-dihydroxyvitamin D3, vitamin D binding protein (VDBP) and FGF-23 within 24 hours of AKI onset and also 5 days later. Bioavailable 25(OH)D and 1,25(OH)2D levels, which are defined as the total of free- and albumin-bound 25(OH)D and 1,25(OH)2D, were calculated using equations. Patients with AKI had lower levels of 1,25(OH)2D and VDBP and similar levels of bioavailable 25(OH)D and 1,25(OH)2D at the time of enrolling. 25(OH)D was inversely associated with severity of sepsis in the overall sample ($P < 0{\cdot}001$). They concluded that Bioavailable 25(OH)D could have a function as a biomarker of adverse outcomes among patients with established AKI and they used FGF-23 as a predictor in their study.

Whether FGF-23 levels are associated with detrimental outcomes in patients with AKI was studied by David E leaf et al[14] in 2012. This study had 30 participants with AKI, and 30 controls from the general hospital wards and ICU. Plasma levels of C-terminal FGF 23 and vitamin D metabolites were noted within 24 hours of AKI onset and 5 days later. The endpoint was taken as death or need for renal replacement therapy (RRT). They found that the FGF-23 levels at enrolling were significantly higher among patients with AKI than controls. FGF-23 at enrolling correlated inversely with 25-hydroxyvitamin D ($P<0.001$) and 1,25-dihydroxyvitamin D ($P=0.003$) and directly with phosphate ($P=0.02$) and PTH ($P=0.005$). Among patients with AKI, FGF-23 at enrolling was significantly associated with the death and RRT, even after adjusting for age and enrolment serum creatinine. They concluded that in

patients with AKI, FGF-23 levels are increased and corelate with greater risk of death or need for RRT.

In 2018 in yet another study by David E Leaf et al[20] studied if FGF-23 was associated with mortality in critically ill. They included 817 patients with AKI requiring RRT who were already subjects in the ARF Trial Network (AT N) study, and 710 patients with and without AKI who were a part in the Validating Acute Lung Injury biomarkers for Diagnosis (VALID) study. In the ATN study, patients in the highest quartile were compared with lowest quartile of C-terminal (cFGF-23) and intact FGF-23 (iFGF-23), they had 3.84- and 2.08-times higher mortality risk, respectively. However, levels of PTH, vitamin D metabolites, calcium, and phosphate were not associated with 60-day mortality. Coming to the VALID study, similarly when patients of highest quartile were compared with lowest quartile of cFGF-23 and iFGF-23, they had 3.52- and 1.93-times higher mortality risk. They inferred that elevated FGF-23 is independently associated with higher mortality in critically ill patients.

David E Leaf et al[18] in 2017 evaluated the relationship and association of elevated urinary/plasma FGF-23 levels and AKI/death in critically ill patients. 350 critically ill patients who were under ICU care were investigated in the study. The primary objective was to check whether higher urinary FGF-23 levels correlated with AKI or in-hospital mortality. On the first day of ICU admission urinary FGF-23 levels were measured. Plasma levels of FGF-23, calcium, phosphate, PTH, and vitamin D metabolites were measured in a subgroup of study sample. The study reflected the statistically significant association between Urinary and plasma FGF-23 levels and AKI/mortality. Prolonged hospital stays, mortality rates at 90 days and 1 year and several other un-favourable outcomes were associated with higher urinary FGF-23 levels the study inferred a significant point that the elevated FGF-23 levels in the urine or plasma can be a potential biomarker of AKI, mortality and other adverse outcomes in ICU.

David E leaf et al[19] set to determine if FGF-23 was increasing in the periods of acute kidney injury following cardiac surgery and were associated with worsening of AKI and death. Serum cFGF-23 concentrations were documented before the surgery, after cardiopulmonary bypass (CPB), and on POD 1 and 3. 18 with AKI and 18 with no AKI as controls were subdivided. iFGF-23, PTH, phosphate, and vitamin D metabolites were also documented. From the end of CPB, cFGF-23 levels were consistently high in AKI subjects. The early rise in cFGF-23 was present before those in rest of the mineral metabolites. iFGF-23 was also elevated in those who had severe AKI, but the raise was lesser than cFGF-23. The study was standardized for age, pre-surgery eGFR, and CPB duration. Higher cFGF-23 levels at the termination of CPB were significantly associated with greater risk of severe AKI and the need for RRT or death. Study showed cFGF-23 levels rise early in AKI following cardiac surgery and are associated with adverse postoperative events.

Oliveira Neves et al. [113] investigated the mechanism of raised FGF-23 in severe AKI and studied if it is mediated by endothelial-glycocalyx related biomarkers. Blood samples were collected from ICU patients on the day of ICU admission. 265 patients were included and 82 developed severe AKI - defined as per KDIGO stage 2/3. Blood samples collected were on day one of ICU admission. After standardization, FGF-23 vascular cell adhesion protein 1 (VCAM-1), angiopoietin 2 (AGPT2), syndecan-1 and intercellular adhesion molecule-1 (ICAM-1) could be corelated with severe AKI. When the 3 biomarkers were included in a directed acyclic graph (DAG), the Bayesian network learning stipulated the following causal pathway FGF-23 → syndecan-1 → VCAM-1 → AGPT2 → severe AKI.

Montasser M Zeid et al[114] in their study tried to determine the association between FGF-23 levels in patients with AKI and morbidity, mortality, and the need for RRT. They had 2 groups: a group of 30 AKI patients [as per the criteria established by Acute Kidney Injury Network

(AKIN) grading of AKI] and another group of 30 healthy controls standardized for age and sex. Plasma levels of cFGF-23, 1,25(OH)2D, and intact PTH were measured within 24 h of AKI onset and also after 5 days. Death or need for RRT were the endpoints. AKI patients had higher FGF-23 levels on day 1 than controls. There was a negative correlation between FGF-23 and vitamin D on day 1 ($p<0.023$), whereas the negative correlation between FGF-23 and vitamin D on day 5 had a P-value of 0.102(not significant). A statistically significant direct correlation between FGF-23 and both APAPCHE and SOFA scores, ($P <0.001$). With a sensitivity of 100% and 85%, FGF-23 was established to be a good predictor of death at a cutoff value of 280 pg/ml.

C-terminal and intact FGF-23 in critical illness and their associations with AKI and in-hospital deaths was studied by Karolina Rygasiewicz et al[115] in 2017. FGF-23 levels were measured within 24 hours of ICU admission. The primary endpoints were AKI and in-hospital death during the ICU stay. 79 patients admitted to ICU. cFGF-23 levels were significantly raised in subjects who had AKI and non-survivors ($p < .001$). ROC analysis of cFGF-23 had an AUC of 0.81 and 0.85 for prediction of new AKI and mortality during ICU stay, respectively. They inferred that cFGF-23 levels could be useful predictor for AKI and death in ICU.

Myles Wolf, et al[116] in their paper, opined that raised FGF-23 is a risk factor for death and allograft loss in renal transplant recipients. When enrolled to the study, estimated GFR was 51 +/- 21 ml/min per 1.73 meter^2 and median cFGF-23 was 28 RU/ml (interquartile range, 20 to 43 RU/ml). Elevated FGF-23 levels were corelated with increased risk of the endpoints i.e. death and allograft loss ($P < 0.001$). However, other indicators of phosphorus metabolism, including serum phosphate and PTH levels, were not consistently associated with endpoints.

They concluded that a raised FGF-23 is an independent risk factor for mortality and allograft loss in renal transplant recipients.

A study was conducted to correlate the FGF-23 levels and detrimental outcomes in AKI by Ahmed Fayed et al[117]. 30 patients with AKI (AKIN criteria) were enrolled and serum FGF-23 levels documented within 24 hours of AKI onset to associate the FGF-23 levels with death and need for RRT. On admission FGF-23 levels were found to be higher in patients who died than in the survivors (P = 0.004, significant). Also, FGF-23 levels in patients who needed RRT were higher than in other patients (P = 0.04, significant). FGF-23 levels and SOFA score were positively corelated (P =0.03).

Timo Speer et al[118] assessed FGF-23 for its predictive ability against an established scoring model. In 859 patients who underwent elective heart surgery and were followed up for a median 822 days post discharge. FGF-23 measured once preoperatively was compared as a prognostic tool with the EuroSCORE II with respect to postoperative mortality in a cardiac surgery, AKI, non-occlusive mesenteric ischemia, hospital course, recovery and long-term course. FGF-23 level corelated significantly with postoperative outcome and complications. The predictive value of FGF-23 for death in the ROC curve was better than the EuroSCORE II. Furthermore, preoperative FGF-23 level independently predicted postoperative AKI and non-occlusive mesenteric ischemia comparably to the EuroSCORE II. FGF-23 could also predict other clinical course parameters, including duration of surgery, ventilation time and duration of hospital stay. They concluded that in patients undergoing elective heart surgeries, a preoperative FGF-23 is a powerful predictor of surgical mortality, postoperative complications and long-term outcome. Its predictive power is very well comparable to the well-established EuroSCORE II.

Julia J. Scialla et al[119] used survival analysis to evaluate whether raised FGF-23 is associated with higher risk of adjudicated congestive heart failure (CHF) and atherosclerotic events in patients with CKD stages 2–4. Patients had a median follow-up of 3.7 years. Demographic characteristics, renal functions, cardiovascular risk factors, and treatments were standardized among the study population. Elevated FGF-23 was independently associated with risk of CHF and atherosclerotic complications. Higher FGF-23 levels were associated more with CHF than with atherosclerotic complications (P=0.02), and consistently associated with increased risk of CHF events across all subgroups. They inferred that elevated FGF-23 was independently associated with higher risk of cardiovascular complications, especially CHF, in patients with CKD stages 2–4.

MATERIALS AND METHODS

Source of data

The present study was carried out at BLDEU B.M PATIL MEDICAL COLLEGE HOSPITAL VIJAYAPUR over a period of 18 months during From December 2018 to May 2020 among the critically ill patients, admitted in the Intensive Care Unit who needed ICU stay of 24 hours or more.

Study design

Prospective cross-sectional study

SAMPLE COLLECTION

Oral and written consent will be taken from the subjects prior to the collection of specimens. Blood sample will be collected on admission to ICU /medical emergency ward. sample will be tested for plasma fibroblastic growth factor 23 by double antibody sandwich enzyme linked immunosorbent one-step process assay. (ELISA).

SAMPLE SIZE:

By using formula:

$n= Z^2 p (1-p)/d^2$

where

Z = z statistic at 5% level of significance (1.96)

d = margin of error

p = anticipated prevalence rate (prevalence of critical illness and admissions to ICU of BLDEU B.M PATIL MEDICAL COLLEGE HOSPITAL VIJAYAPUR over last 3 years= 70%)

A sample size of 72 subjects will allow the study to assess the plasma FGF-23 among critically ill patients. 90% confidence level and margin of error of 10% and 5% expected loss/missing data. with finite population correction.

Selection criteria

Inclusion criteria

1. Patients above 18 years
2. Admitted in ICU for a minimum of 24 hours.

Exclusion criteria

1. Patients with chronic kidney disease
2. Any patient who dies within 24 hours of admission to ICU.

METHOD OF COLLECTION OF DATA

The selected patient's informed consent was taken from the relative of the patient explaining the nature of the study and the risks involved in the participation for this study. Prospectively collected data of patients, who were admitted to ICU over 18 months period, was reviewed. The study included patients with varied etiologies. Any decision for mechanical ventilation was taken by the treating intensivist. Clinical and demographic profile at the time of admission to ICU including age, sex, smoking status, history of previous hospital admissions, associated chronic illnesses like hypertension, diabetes mellitus, chronic obstructive pulmonary disease was recorded. A careful and detailed history was recorded and thorough clinical examination

was conducted. All the points mentioned in the proforma were recorded. Additional information if any was recorded. Total blood counts, renal functions, liver functions and serum FGF 23 levels done at the time of admission were also recorded. Chest X-ray and arterial blood gas analysis were obtained. Days on ventilator, days of ICU stay and days of hospital stay were recorded for all the patients. Duration of mechanical ventilation was defined as the time elapsed from the initiation of ventilatory support to the onset of weaning. The onset of weaning was the time that the physician in charge considered the patient likely to resume and sustain spontaneous breathing. Weaning was performed by either a reduction in the level of ventilator support or a trial of spontaneous breathing. Mechanical ventilation was delivered through an orotracheal tube and/or a tracheostomy.

The patients were followed up till their stay in hospital. They qualified for the study if they were in ICU for 24 hours or more. They were excluded from the study if they were discharged from ICU before 24 hours or died within 24 hours of admission.

For patients who were included in the study, FGF-23 levels were measured using double sandwich enzyme linked immunosuppressant one step process assay. Adding standard, test sample and HRP- labelled fibroblast growth factor-23 antibodies to enzyme wells which are precoated with fibroblast growth factor 23 antibody, and carrying out incubation and washing to remove the uncombined enzyme. Upon adding chromogen solution, A and B, the color of the liquid will change into blue and the reaction with the acid will cause the color to become yellow. The depth of the color and the concentration of the fibroblast growth factor-23 sample are positively corelated and corresponding concentration obtained by ELISA reader. This test was done on the day of admission to ICU.

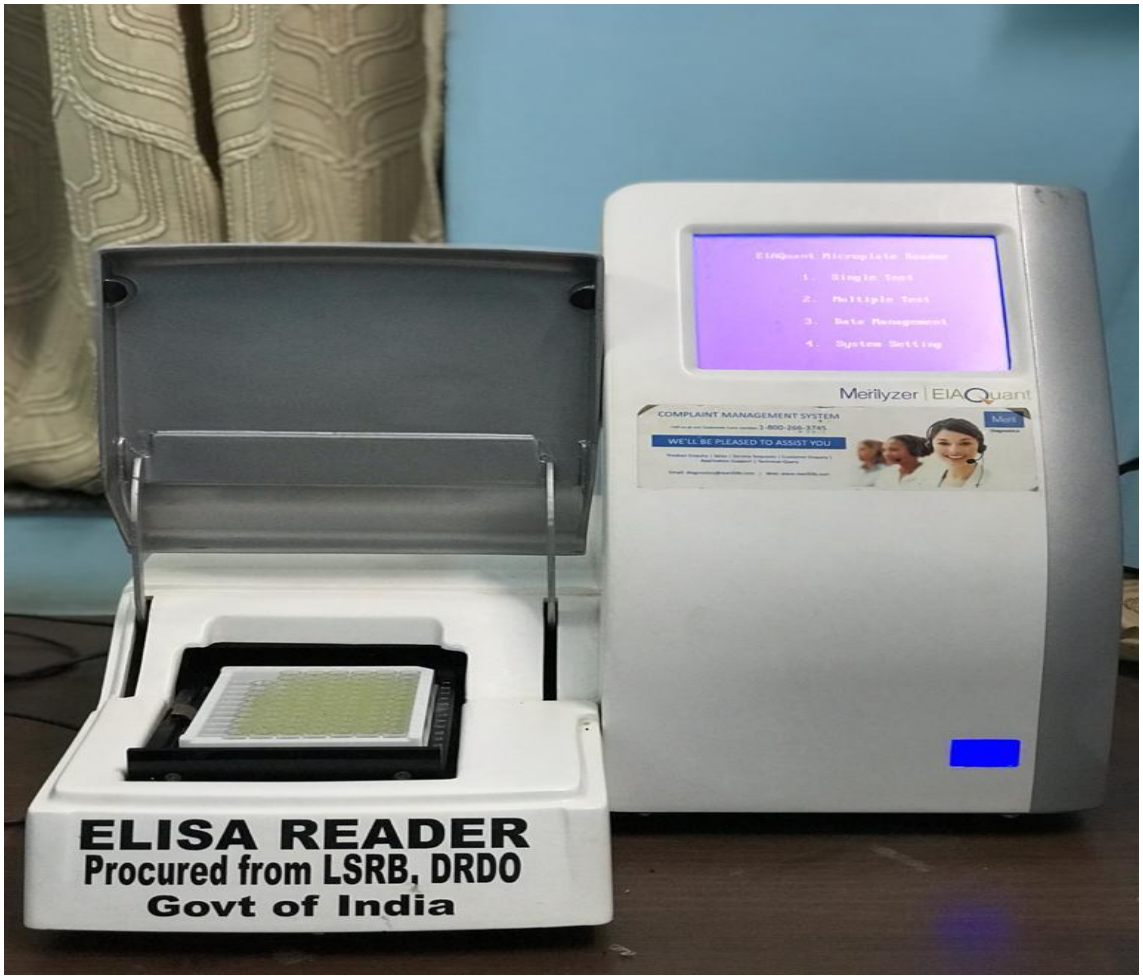


Figure 5 and 6 FGF 23 Elisa reader with reagent.(procured from QAYEE BIO)

Acute physiology and chronic health evaluation II (APACHE II) score which depends on different clinical and laboratory variables was calculated on the day of ICU admission using the worst value in the first 24 h mainly to predict the mortality.

Table 2: Acute Physiologic and Chronic Health Evaluation (APACHE II) Scoring System[3]

Physiologic variables									point score
	+4	+3	+2	+1	0	+1	+2	+3	+4
1.Temperature(°C)	≥41 °	39°-40.9 °	-	38.5°-38.9°	36°-38.4 °	34°-35.9 °	32°-33.9 °	30°-31.9 °	<29.9 °
2.Mean arterial pressure (mm Hg)	≥160	130-159	110-129	-	70-109	-	50-69	-	<49
3.Heart rate	≥180	140-179	110-139	-	70-109	-	55-69	40-54	<39
4.Respiratory rate	≥50	35-49	-	25-34	12-24	10-11	6-9	-	<5
5.Oxygenation; a) Fio2 ≥0.5; useA-aDO2 b) Fio2<0.5; use pao2(mmHg)	≥500 -	350-499 -	200-349 -	- -	<200 >70	- 61-70	- -	- 55-60	- <55

6.Arterial PH	≥7.7	7.6-7.69	-	7.5-7.59	7.33-7.49	-	7.25-7.32	7.15-7.24	<7.15
7.Serum Na(mmol/L)	≥180	160-179	155-159	150-154	130-149	-	120-129	111-119	<110
8.Serum K(mmol/L)	≥7	6-6.9	-	5.5-5.9	3.5-5.4	3-3.4	2.5-2.9	-	<2.5
9.Serum creatinine(mg/dl); double point score for acute renal failure	≥3.5	2-3.4	1.5-1.9	-	0.6-1.4	-	<0.6	-	-
10.Hct (%)	≥60	-	50-59.9	46-49.9	30-45.9	-	20-29.9	-	<20
11.WBC(in1000s)	≥40	-	20-39.9	15-19.9	3-14.9	-	1-2.9	-	<1

12.Glassgow coma scale (GCS)	Score=15 minus actual GCS (see table Glasgow coma scale) EYE OPENING; Spontaneous 4 To Speech 3 To pain 2 No response 1 VERBAL RESPONSE; Fully oriented 5 Mild confusion 4 Moderate confusion(inappropriate) 3 Severe confusion(incomprehensible) 2 No response 1 MOTOR RESPONSE; Obeys commands 6 Localises pain 5 Withdrawal to pain 4 Abnormal flexor response 3 Extensor response 2 No response 1

Add

0 points for age <44;

2 points, 45–54 yr;

3 points, 55–64 yr;

5 points, 65–74 yr;

6 points ≥ 75 yr

Table 3: Approximated in-hospital mortality rates- from Knaus et al [120]

APACHE II Score	Nonoperative	Postoperative
0-4	4%	1%
5-9	8%	3%
10-14	15%	7%
15-19	25%	12%
20-24	40%	30%
25-29	55%	35%
30-34	73%	73%
>34	85%	88%

Statistical analysis

All characteristics were summarized descriptively. For continuous variables, the summary statistics of mean± standard deviation (SD) were used. For categorical data, the number and percentage were used in the data summaries and diagrammatic presentation. Chi-square (χ^2) test was used for association between two categorical variables.

The formula for the chi-square statistic used in the chi square test is:

$$\chi_c^2 = \sum \frac{(O_i - E_i)^2}{E_i}$$

The subscript “c” are the degrees of freedom. “O” is observed value and E is expected value. C= (number of rows-1)* (number of columns-1)

The difference of the means of analysis variables between two independent groups was tested by unpaired t test.

The t statistic to test whether the means are different can be calculated as follows:

$$t = \frac{(\overline{x_1} - \overline{x_2}) - (\mu_1 - \mu_2)}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

where $\bar{x}_1$ = mean of sample 1

$\bar{x}_2$ = mean of sample 2

n_1 = number of subjects in sample 1

n_2 = number of subjects in sample 2

s_1^2 = variance of sample 1 = $\frac{\Sigma(x_1 - \bar{x}_1)^2}{n_1}$

s_2^2 = variance of sample 2 = $\frac{\Sigma(x_2 - \bar{x}_2)^2}{n_2}$

ROC analysis for Sensitivity- specificity was done to check relative efficiency.

Logistic linear regression analysis was employed to assess the adjusted effect of determinants of categorical study variable. The logistic regression equation is expressed as:

$$logit(p) = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + \ldots + b_kX_k$$

where p is the probability of presence of the characteristic of interest. The logit transformation is defined as the logged odds:

$$odds = \frac{p}{1-p} = \frac{probability\ of\ presence\ of\ characteristic}{probability\ of\ absence\ of\ characteristic}$$

and

$$logit(p) = \ln\left(\frac{p}{1-p}\right)$$

If the p-value was < 0.05, then the results were considered to be statistically significant otherwise it was considered as not statistically significant. Data were analysed using SPSS software v.23 (IBM Statistics, Chicago, USA) and Microsoft office 2007.

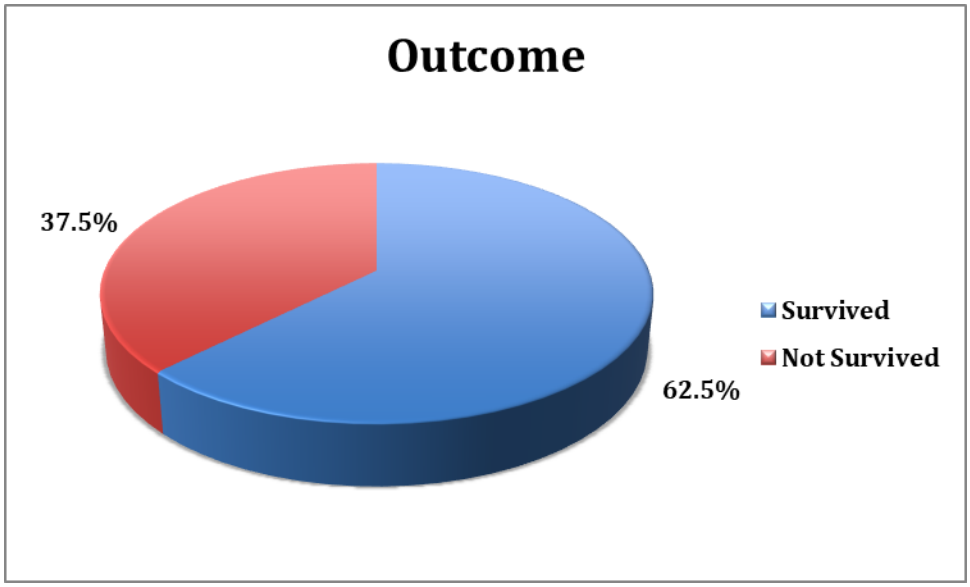
RESULTS

The present study was conducted on 72 patients who were critically ill and required ICU care for 24 hours or more. The study was done over period of 18 months during December 2018 to May 2020.

Table 4: Distribution of Cases according to Outcome

Outcome	N	%
Survived	45	62.5
Not Survived	27	37.5
Total	72	100

Graph 1: Distribution of Cases according to Outcome

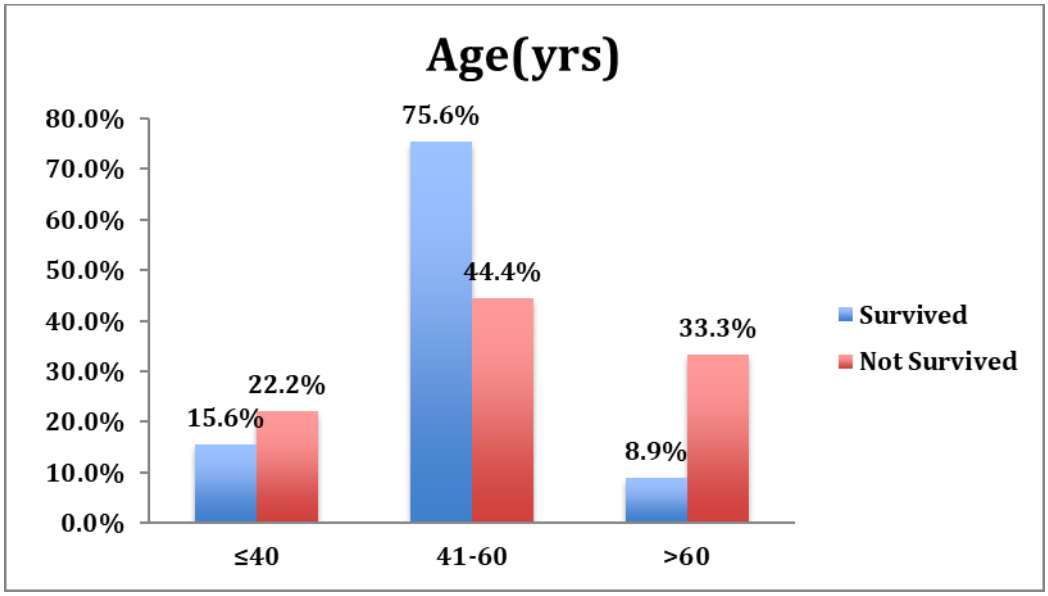


72 patients were included in study, out of which 45 (62.5%) patients were discharged from the hospital (survivors) and 19 (37.5%) died in ICU (non-survivors)

Table 5: Distribution of Age between Study Groups

Age(yrs)	Survived		Not Survived		p value
	N	%	N	%	
≤40	7	15.6%	6	22.2%	0.014*
41-60	34	75.6%	12	44.4%	
>60	4	8.9%	9	33.3%	
Total	45	100.0%	27	100.0%	

Graph 2: Distribution of Age between Study Groups

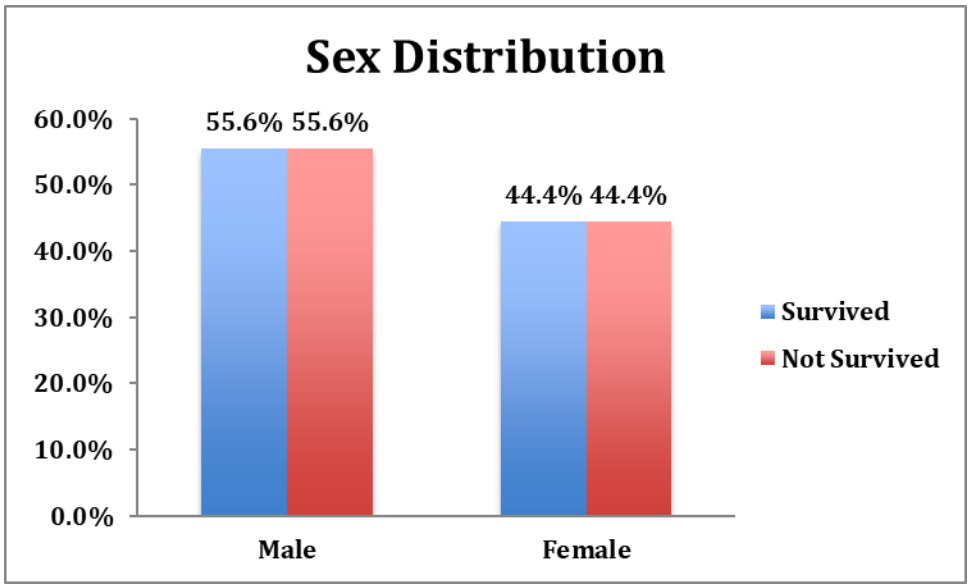


The mean age of study population was 51.9 years . The mean age in survivors was 50.2 years with a range of 22-76 years. The mean age in non-survivors was 54.6 years with a range of 32-76 years. There was a significant difference (p = 0.014) between the two groups indicating a higher age at admission for non-survivors.

Table 6: Distribution of Sex between Study Groups

Sex	Survived		Not Survived		p value
	N	%	N	%	
Male	25	55.6%	15	55.6%	0.999
Female	20	44.4%	12	44.4%	
Total	45	100.0%	27	100.0%	

Graph 3: Distribution of Sex between Study Groups

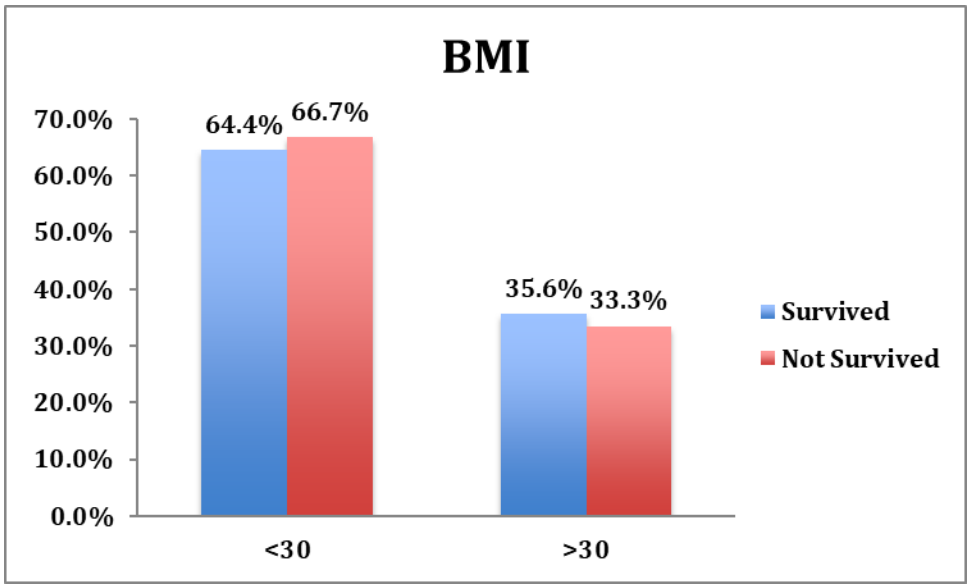


Study population had 55.6% males and 44.4% females. Amongst survivors 25 (55.6%) were males and 20 (44.4 %) were females. In non-survivors 15 (55.6 %) were males and 12 (44.4 %) were females. There was no significant difference between males and females in the study, and they were equally affected critically.

Table 7: Distribution of BMI between Study Groups

BMI	Survived		Not Survived		p value
	N	%	N	%	
<30	29	64.4%	18	66.7%	0.848
>30	16	35.6%	9	33.3%	
Total	45	100.0%	27	100.0%	

Graph 4: Distribution of BMI between Study Groups



Study population had 62.5 % patients with BMI below 30 and 37.5% were obese. Amongst survivors 29 (64.4 %) were non obese and 16 (35.6 %) were obese. In non-survivors 18 (66.7 %) were obese and 9 (33.3 %) were non-obese. There was no significant difference between BMI distribution in both the groups.

Table 8: Distribution of Type of Comorbidities between Study Groups

Type of Comorbidities	Survived		Not Survived		p value
	N	%	N	%	
DM	17	37.8%	14	51.9%	0.243
HTN	22	48.9%	14	51.9%	0.808
IHD	9	20.0%	8	29.6%	0.352

Graph 5: Distribution of Type of Comorbidities between Study Groups

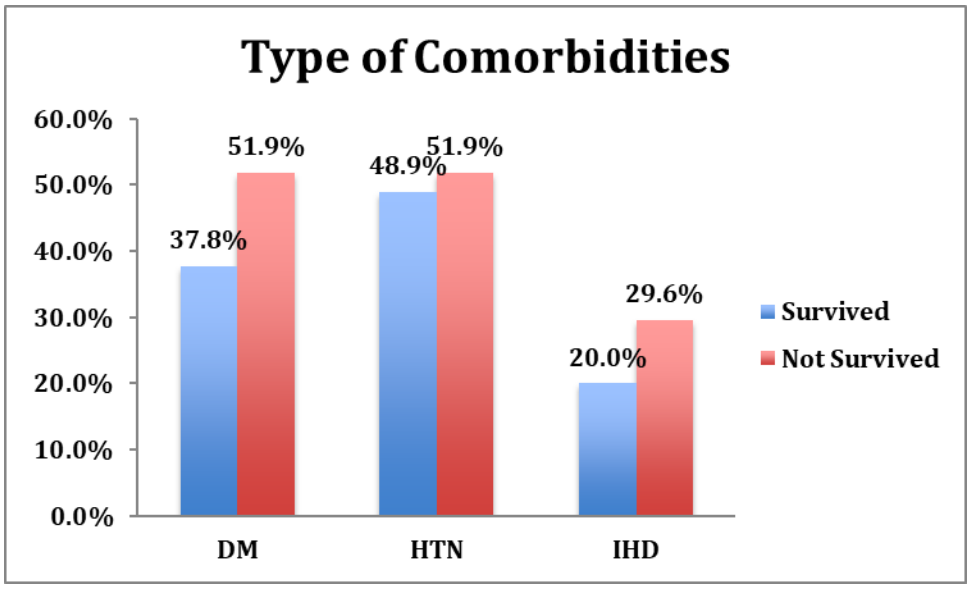
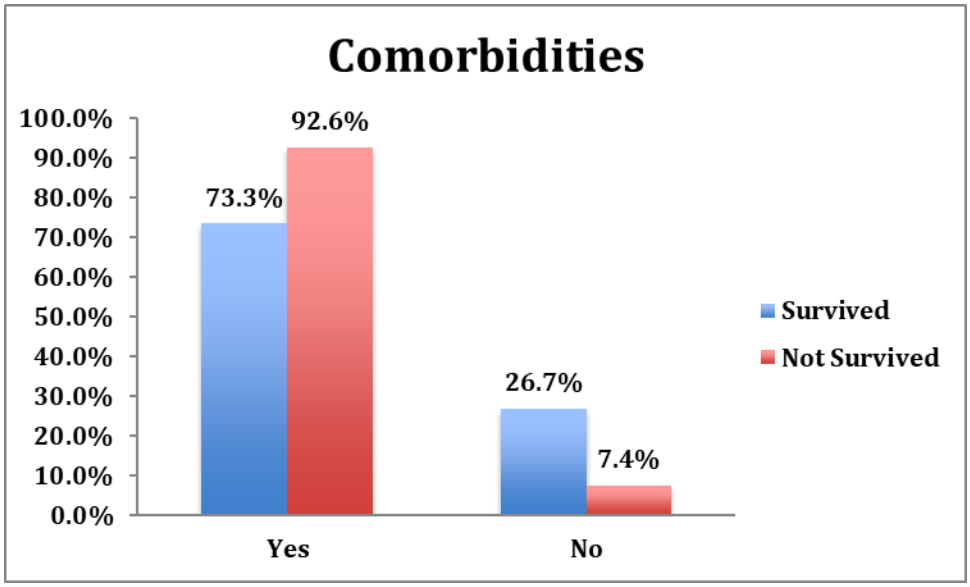


Table 9: Distribution of Comorbidities between Study Groups

Comorbidities	Survived		Not Survived		p value
	N	%	N	%	
Yes	33	73.3%	25	92.6%	0.046*
No	12	26.7%	2	7.4%	
Total	45	100.0%	27	100.0%	

Note: * significant at 5% level of significance (p<0.05)

Graph 6: Distribution of Comorbidities between Study Groups

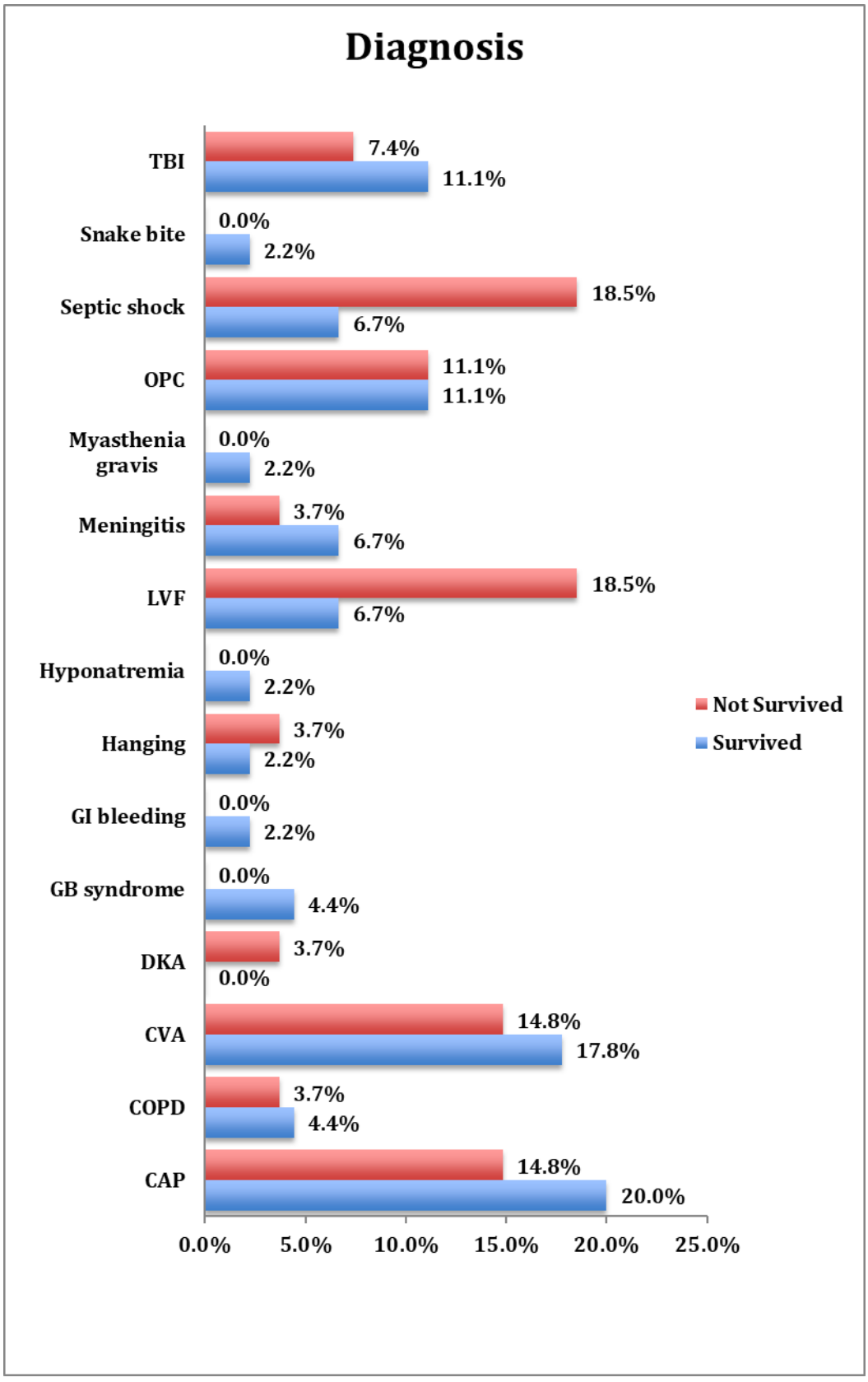


80.5 % patients had comorbidities like diabetes mellites, hypertension and ischemic heart disease. Amongst survivors 73.3% and 92.6 % of non-survivors had comorbidities. There was a statistical significance indicating patients with comorbidities were more likely to not survive.

Table 10: Distribution of Diagnosis between Study Groups

Diagnosis	Survived		Not Survived		p value
	N	%	N	%	
CAP	9	20.0%	4	14.8%	0.722
COPD	2	4.4%	1	3.7%	
CVA	8	17.8%	4	14.8%	
DKA	0	0.0%	1	3.7%	
GB syndrome	2	4.4%	0	0.0%	
GI bleeding	1	2.2%	0	0.0%	
Hanging	1	2.2%	1	3.7%	
Hyponatremia	1	2.2%	0	0.0%	
LVF	3	6.7%	5	18.5%	
Meningitis	3	6.7%	1	3.7%	
Myasthenia gravis	1	2.2%	0	0.0%	
OPC	5	11.1%	3	11.1%	
Septic shock	3	6.7%	5	18.5%	
Snake bite	1	2.2%	0	0.0%	
TBI	5	11.1%	2	7.4%	
Total	45	100.0%	27	100.0%	

Graph 7: Distribution of Diagnosis between Study Groups

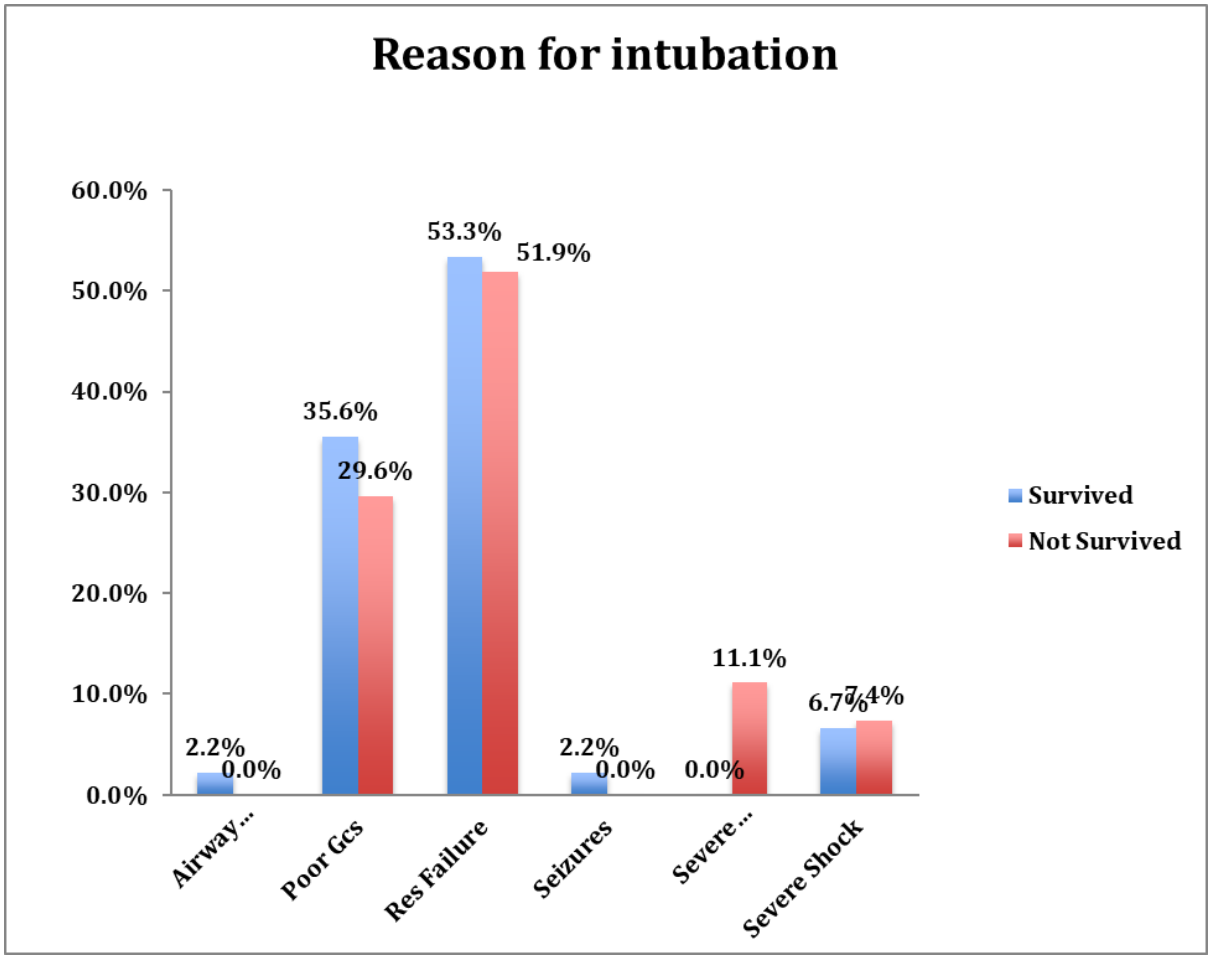


Out of the 72 cases in study group, largest number of patients were with, Neurological disorders, comprising 26 cases (36.1%). These included Cerebro Vascular Accident (CVA) 12 Cases, Traumatic brain injury 7 cases, Guillain Barre syndrome 2 cases, Meningitis 4 Cases, and myasthenia gravis 1 Case. Respiratory disorders were 16 cases (22.2%) with 3 cases of COPD and 13 cases of community acquired pneumonia. 8 cases (11.1%) were Organophosphorous compound poisoning. Metabolic causes 2 cases (2.7%) included 1 case of Hyponatremic metabolic encephalopathy and Diabetic ketoacidosis each. Left ventricular failure 8 cases (11.1%), Septic shock 8 cases (11.1%), Gastrointestinal bleed 1 case (1.4%) snake bite 1 case (1.4%), partial Hanging 1 case (1.4%) comprised the rest of the cases.

Table 11: Distribution of Reason for intubation between Study Groups

Reason for intubation	Survived		Not Survived		p value
	N	%	N	%	
Airway Protection	1	2.2%	0	0.0%	0.269
Poor GCS	16	35.6%	8	29.6%	
Respiratory Failure	24	53.3%	14	51.9%	
Seizures	1	2.2%	0	0.0%	
Severe Acidosis	0	0.0%	3	11.1%	
Severe Shock	3	6.7%	2	7.4%	
Total	45	100.0%	27	100.0%	

Graph 8: Distribution of Reason for intubation between Study Groups



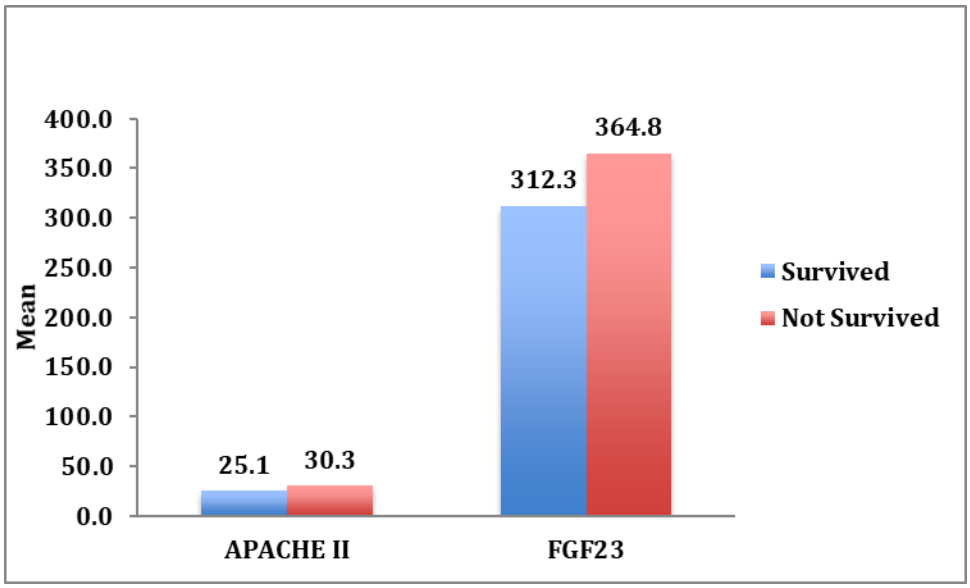
In our study, 52.7% patient had to be put on mechanical ventilation secondary to respiratory failure because of ARDS, acute pulmonary edema and aspiration pneumonia. 33 % patients had to be put on mechanical ventilation secondary to poor GCS, 7% were intubated in view of severe shock, 4% because of severe acidosis. 2 patients were intubated because of airway protection in GI bleed and seizures each.

Table 12: Mean APACHE II and FGF23 between Study Groups

Parameters	Survived		Not Survived		p value
	Mean	SD	Mean	SD	
APACHE II	25.1	4.7	30.3	4.2	<0.001*
FGF23	312.3	50.9	364.8	109.6	0.007*

Note: * significant at 5% level of significance (p<0.05)

Graph 9: Mean APACHE II and FGF23 between Study Groups



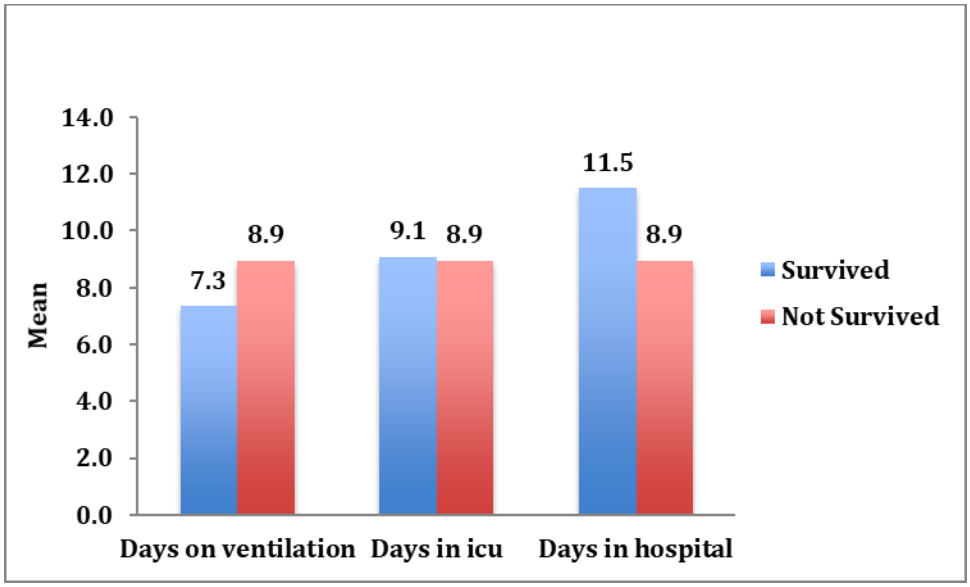
The mean levels of FGF23 and APACHE scores on day one was 331.99 and 27.04 respectively. In survivors, they were 312.3 and 25.1 respectively. In non-survivors, they were 364.8 and 30.3 respectively. Both of which was significantly higher (p=0.007 and p< 0.003 respectively) in non-survivors.

Table 13: Mean duration between Study Groups on ventilator/ ICU /Hospital stay.

Parameters	Survived		Not Survived		p value
	Mean	SD	Mean	SD	
Days on ventilation	7.3	2.7	8.9	5.2	0.090
Days in ICU	9.1	2.8	8.9	5.2	0.882
Days in hospital	11.5	3.4	8.9	5.2	0.013*

Note: * significant at 5% level of significance (p<0.05)

Graph 10: Mean days between Study Groups



In the study group, the mean duration of mechanical ventilation was 7.9 Days. The average number of days survivors were on mechanical Ventilation was 7.3 days (+/- 2.7 days) and in non-survivors, this duration was 8.9 Days (+/- 5.2 days). The duration of ICU stay was not found to be significantly different in (p = 0.882) in the two groups.

In the study group, the mean duration of ICU was 9.01 Days. The average number of days survivors were in ICU was 9.1 days (+/- 2.8 days) and in non-survivors, this duration was 8.9

Days (+/- 5.2 days). The duration of mechanical ventilation was not found to be significantly different in (p = 0.090) in the two groups.

In the study group, the mean duration of hospital stay was 10.52 Days. The average number of days survivors were in hospital was 11.5 days (+/- 3.4 days) and in non-survivors, this duration was 8.9 days (+/- 5.2 days). The duration of Hospital stay was found to be significantly higher in (p = 0.013) survivors.

Table 14: Logistic regression results for non-survival

Predictors	**B**	**S.E.**	**Odds ratio**	**p value**
APACHE II	0.242	0.07	1.274	0.001*
FGF23	0.008	0.004	1.008	0.049*
Constant	-9.725	2.472	0	<0.001*

Note: * significant at 5% level of significance (p<0.05)

	-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square
Model Summary	71.981	0.276	0.377

Non-survival prediction from APACHE II and FGF 23 levels were both statistically significant. The above table shows the Logistic regression results for likelihood of non-survival as dependent variable. It was found that the likelihood of non-survival was 27.4% higher with increase in APACHE II score while there was 0.8% more chance of mortality with increase in FGF23 score. The effect of APACHE II and FGF23 score was found to be statistically significant on survival

Table 15: Diagnosis of cases according to FGF23 level among non-survivors

Diagnosis	FGF23						p value
	<300		300-400		>400		
	N	%	N	%	N	%	
CAP	2	20.0%	0	0.0%	2	25.0%	
COPD	0	0.0%	1	11.1%	0	0.0%	
CVA	1	10.0%	2	22.2%	1	12.5%	
DKA	0	0.0%	1	11.1%	0	0.0%	
Hanging	0	0.0%	1	11.1%	0	0.0%	
LVF	2	20.0%	2	22.2%	1	12.5%	0.445
Meningitis	0	0.0%	1	11.1%	0	0.0%	
OPC	2	20.0%	0	0.0%	1	12.5%	
Septic shock	1	10.0%	1	11.1%	3	37.5%	
TBI	2	20.0%	0	0.0%	0	0.0%	
Total	10	100.0%	9	100.0%	8	100.0%	

The non-survivor predictability of FGF23 was not varying with different diagnosis, statistically insignificant difference noted.

Graph 11: Diagnosis of cases according to FGF23 level among non-survivors

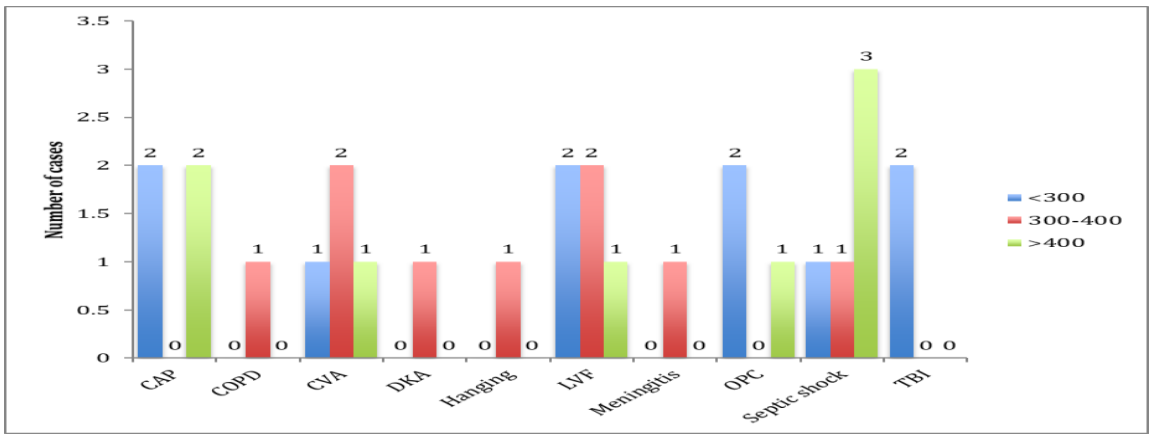


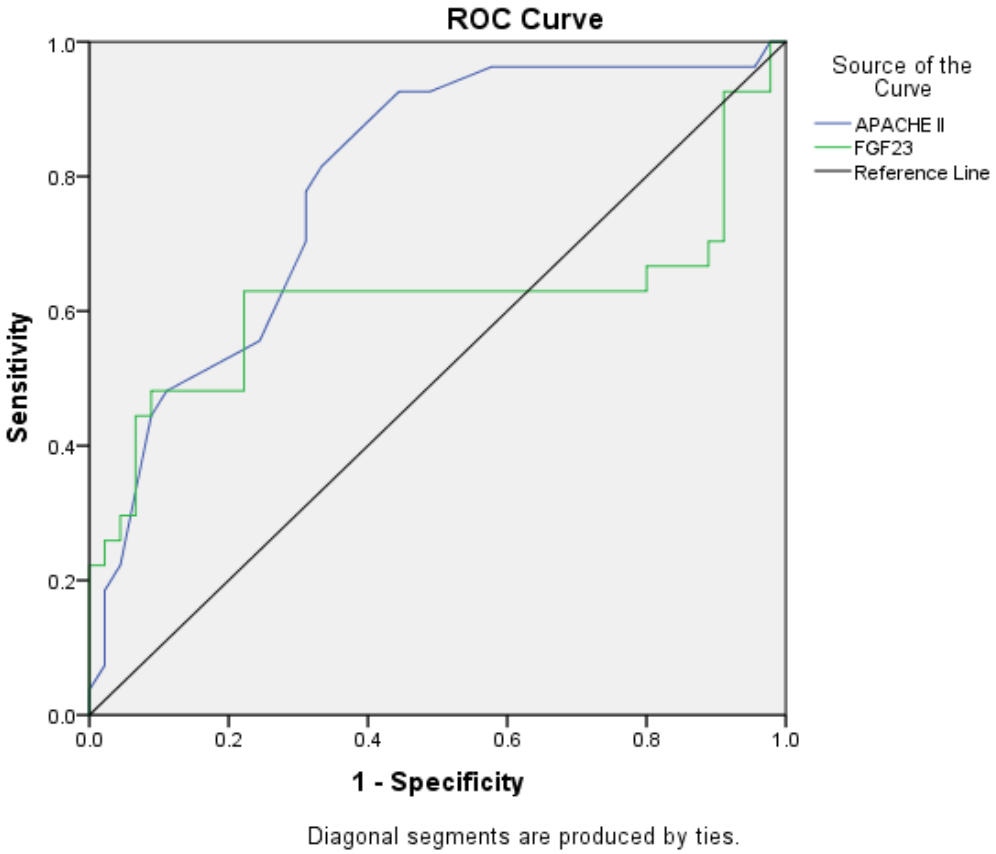
Table 16: ROC Analysis of Scores in Predicting Mortality

Parameters	Area Under the Curve	Std. Error	p value	95% Confidence Interval	
				Lower	Upper
APACHE II	0.789	0.055	<0.001 *	0.682	0.897
FGF23	0.614	0.082	0.107	0.453	0.775

Note: * significant at 5% level of significance (p<0.05)

Parameters	Cut-off value	Sensitivity	Specificity
APACHE II	28.5	70%	69%
FGF23	324.8	63%	62%

Graph 12: ROC Curve of Scores in Predicting Mortality



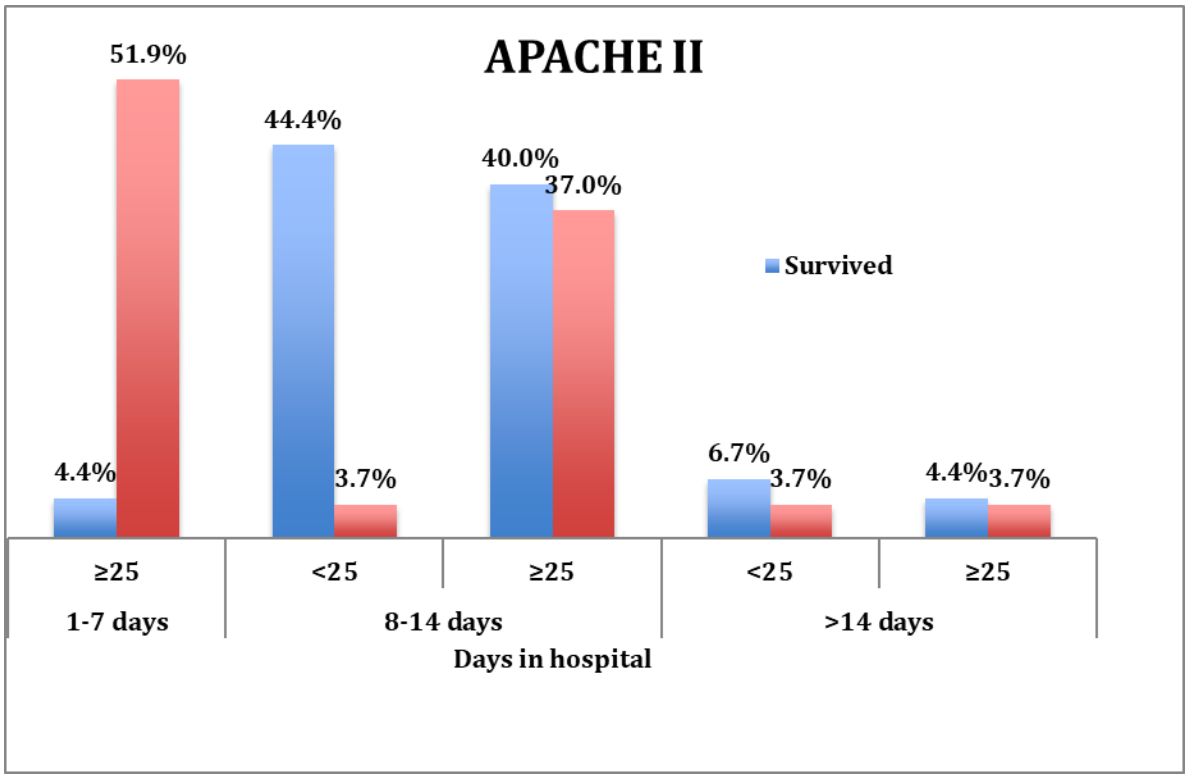
Both APACHE and FGF 23 levels could predict the non-survivors. At a cut off score of 28.5 APACHE II was 70 % sensitive and 69% specific in predicting mortality in ICU. FGF 23 levels at a cut off of 324.8 was 63% sensitive and 62% sensitive in predicting ICU death.

Table 17: Mean APACHE II between Study Groups according to Days in hospital

Days in hospital	APACHE II	Survived		Not Survived		p value
		N	%	N	%	
1-7 days	≥25	2	4.4%	14	51.9%	0.010*
8-14 days	<25	20	44.4%	1	3.7%	0.809
	≥25	18	40.0%	10	37.0%	
>14 days	<25	3	6.7%	1	3.7%	0.748
	≥25	2	4.4%	1	3.7%	
Total		45	100.0%	27	100.0%	

Note: * significant at 5% level of significance (p<0.05)

Graph 13: Mean APACHE II between Study Groups according to Days in hospital

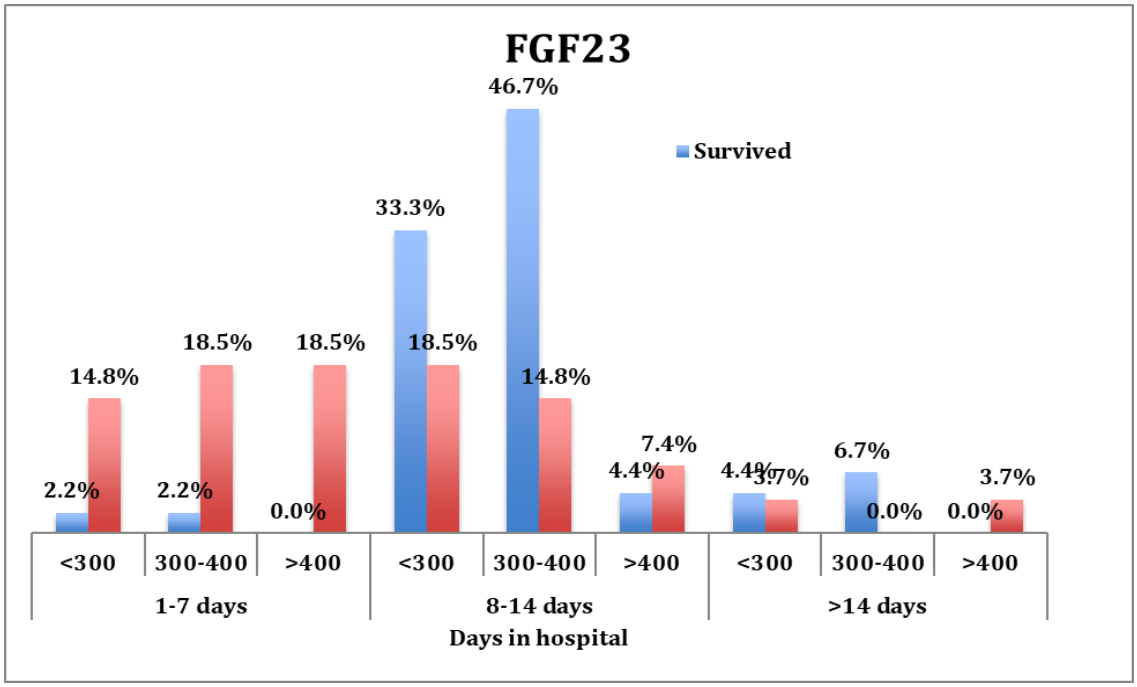


When APACHE II was compared for association with length of hospital stay, it did not give a significant difference between the two groups.

Table 18: Mean FGF23 between Study Groups according to Days in hospital

Days in hospital	FGF23	Survived		Not Survived		p value
		N	%	N	%	
1-7 days	<300	1	2.2%	4	14.8%	
	300-400	1	2.2%	5	18.5%	
	>400	0	0.0%	5	18.5%	0.587
8-14 days	<300	15	33.3%	5	18.5%	
	300-400	21	46.7%	4	14.8%	
	>400	2	4.4%	2	7.4%	0.299
>14 days	<300	2	4.4%	1	3.7%	
	300-400	3	6.7%	0	0.0%	
	>400	0	0.0%	1	3.7%	0.155
Total		45	100.0%	27	100.0%	

Graph 14: Mean FGF23 between study groups according to days in hospital



FGF 23 also did not show any significant difference in association with hospital stay duration between the two groups.

DISCUSSION

The present study was conducted on 72 patients who were critically ill and required ICU stay of 24 hours or more. Serum FGF23 levels were measured as a prognostic marker to predict their outcome as either death in the hospital or discharge from the hospital. Our study also compared the duration of mechanical ventilation, the length of ICU stay and length of hospital stay between survivors and non-survivors.

AGE

In our study, the mean age of study population was 51.9 years. The mean age in survivors was 50.2 years with a range of 22-76 years. The mean age in non-survivors was 54.6 years with a range of 32- 76 years. There was a significant difference ($p = 0.014$) between the two groups indicating a higher age at admission for non-survivors. One study[121] reports the age of patients to be critically ill and requiring ventilator as 59.2 (+/- 17.3) years. Another study[122] reports the age of non-survivors as 58 years (+/- 3.8 years) which was significantly more than survivors 48 years ($p<0.05$). These findings are similar to our study.

SEX

Our study included 40 males (55.6%) and 32 females (44.4%). Amongst 45 survivors 25 (55.6%) were males and 20 (44.4%) were females. In 27 non-survivors 15 (55.6%) were males and 12 (44.4 %) were females. There was no significant difference between males and females in the study with regard to the outcome. However, in few studies [121,122 males] were found to be more likely to be suffer from critical illness. In one study[121], it was found to be 59.3% males and 38.7% females. Another study[122] reported it to be 57% males and 43% females.

COMORBIDITIES

80.5 % patients had comorbidities like diabetes mellites, hypertension and ischemic heart disease. Amongst survivors 73.3 % and 92.6 % of non-survivors had comorbidities. There was a statistical significance indicating patients with comorbidities were more likely to not survive.

ETIOLOGICAL DIAGNOSIS OF PATIENTS AT ADMISSION TO MICU

Our study included 72 cases of varied etiologies. Out of the 72 cases largest number of patients were with, Neurological disorders, comprising 26 cases (36.1%). These included Cerebro Vascular Accident (CVA) 12 Cases (16.6%), Traumatic brain injury 7 cases (9.7%), Guillain Barre syndrome 2 cases (2.7%), Meningitis 4 Cases (5.6%), and myasthenia gravis 1 Case (1.3%). Respiratory disorders were 16 cases (22.2%) with 3 cases of COPD (4.1%) and 13 cases of community acquired pneumonia (18%). 8 cases (11.1%) were Organophosphorous compound poisoning. Metabolic causes 2 cases (2.7%) included 1 case of Hyponatremic metabolic encephalopathy and Diabetic ketoacidosis each. Left ventricular failure 8 cases (11.1%), Septic shock 8 cases (11.1%), Gastrointestinal bleed 1 case (1.4%) snake bite 1 case (1.4%), partial Hanging 1 case (1.4%) comprised the rest of the cases.

Our study suggests that the largest number of patients requiring mechanical ventilation had neurological disease as the etiology.

REASON FOR THE INITIATION OF MECHANICAL VENTILATION

In our study, 52.7% patient had to be put on mechanical ventilation secondary to respiratory failure because of ARDS, acute pulmonary edema and aspiration pneumonia. 33 % patients had to be put on mechanical ventilation secondary to poor GCS, 7% were intubated in view of severe shock, 4% because of severe acidosis. 2 patients were intubated because of airway protection in GI bleed and seizures each. Respiratory failure and poor GCS were the leading causes of intubation in our study.

One study reports 68.8% patients as having acute respiratory failure with acute respiratory distress syndrome, 16.7% acute respiratory failure with coma, 12.8% acute respiratory failure

on chronic pulmonary disease and 1.8% acute respiratory failure with neuromuscular disease.[121] This is comparable to our study.

DIVISION OF PATIENTS BASED ON OUTCOME AT THE END OF STUDY

Our study included 72 patients. Out of these, 45 patients (62.5%) were discharged from the hospital (survivors) and 27 patients (37.5%) expired in the hospital (non-survivors). One similar study[121] has reported 70% survivors and 30% non-survivors. One another study[122] reported 54% survivors and 46% non-survivors. This is comparable to our study. However, in study by Fayed A[117] 40% survived and 60 % patients died. They had a smaller sample size of 30.

SERUM FGF23 LEVELS AS PROGNOSTIC MARKER

In our study, mean FGF 23 level on day of admission (Day 1) for the study group was 332. In survivors, it was 312.3 (+/-50.9) and in non-survivors it was 346.8 (+/- 109.6). It was significantly higher (p 0.007) in non-survivors. Based on the FGF cut off value of 324.8 derived from ROC, 28 patients out of 45 patients in the survivor group had lesser score than the cut off 324.8. 17 patients out of 27 in the non-survivor group had FGF score above the cut off. This indicates higher scores were associated with increased mortality and lesser scores were associated with good survival rate.

Fayed A et al[117] report survivors had lower FGF23(59.3+/- 17.1) values than non-survivors (544.2+/- 75.8) on admission ($p<0.004$). Another study[118] reports similar

findings with non-survivors having higher mean FGF23 levels ($p<0.001$) compared to survivors. In another study [19] higher FGF 23 values were associated with higher death and RRT with odds ratio of 2.25. David E Leaf [20] compared two large cohort studies and identified plasma FGF23 as an independent predictor of mortality. Almost all of the studies available

regarding FGF23 and critical illness have identified it to be having significant association with mortality and other complications. These findings are in congruence with our study.

DURATION OF MECHANICAL VENTILATION

In our study group, the mean duration of mechanical ventilation was 7.4 days. In the survivors, this duration was 7.3 days as compared to non-survivors in which it was 8.9 day (+ 2.9 days). There was no significant difference between survivors and non-survivors (p=0.990). One study[121] reports this duration for all reason for the initiation of mechanical ventilation to be 5.9 days (+ 7.2 days). Another study[123] reports this duration to be 10.5 +/- 10 days. The difference may be observed because of the smaller sample size of our study. This study also reports that FGF23 levels on ICU admission was not a predictor of the length of time spent receiving mechanical ventilation.

DURATION OF ICU STAY

In our study, the patients spent an average of 9.1 days in Intensive Care Unit. This duration was 9.1 days (+/- 2.8 days) in survivors and 8.9 days (+/- 5.2 days) for non-survivors. The length of ICU stay was not significantly different (p = 0.882) in non-survivors and survivors. One study[121] reports length of ICU stay for mechanically ventilated patients to be 11.2 days (+ 13.7 days). This difference is noted presumably because of a larger sample size, i.e. 5183 patients in the study versus 72 patients in our study. This study also reports that FGF23 levels on ICU admission was not a predictor of the length of time spent in ICU in both the groups.

DURATION OF HOSPITAL STAY

In our study group, patients spent a mean of 11.5 days in hospital. Survivors spent 11.5 days (+/- 3.4 days) in hospital whereas non-survivors spent 8.9 days (+/- 5.2 days) in hospital. The average duration of hospital stay was significantly longer (p = 0.013) in survivors. One study[121]

reports an average of 22.5 days (23.7 days) as length of stay in hospital for mechanically ventilated patients. This difference observed can again be attributed to the significantly larger sample size in that study. Longer duration in the survivors can be attributed to the longer course of rehabilitation and recovery that survivors undergo in ward.

COMPARING THE EFFICACY OF FGF 23 IN PREDICTING MORTALITY WITH THAT OF APACHE II SCORES.

APACHE II has been the most widely used mortality predictor score in critical care setting. In our study, mean APACHE II score on day of admission (Day 1) for

the study group was 25.1. In survivors, it was 25.1 (+/- 4.7) and in non-survivors it was 30.3 (+/- 4.2). It was significantly higher ($p < 0.001$) in non-survivors.

Based on the FGF cut off value of 28.5 derived from ROC, only 14 patients out of 45 patients in the survivor group had lesser score than the cut off 28.5. 19 patients out of 27 in the non-survivor group had APACHE II score above the cut off. This indicates higher scores were associated with increased mortality and lesser scores were associated with good survival rate. In our study, area under the curve in ROC analysis was more for APACHE II with 0.789 when compared to 0.614 of FGF levels. Though mortality prediction from APACHE II and FGF 23 levels were both statistically significant, it was found that the likelihood of non-survival was 27.4% higher with increase in APACHE II score while there was 0.8% more chance of mortality with increase in FGF23 score.

CONCLUSIONS AND RECOMMENDATIONS

The research for an ideal, cost effective and simple prognostic indicator for critically ill patients is ongoing. Current cost of FGF 23 analysis kit in India is approximately 39000 rupees and the reagents need to be replenished after 100 uses. FGF 23 is increasingly studied in association with CKD and recently as a prognostic indicator for critically ill. As per our study FGF 23 levels were able to predict mortality (sensitivity 63%, specificity 62%), but APACHE II was found to be superior to FGF 23 in predicting mortality (sensitivity 70%, specificity 69%) and it can be calculated in any patient using the routinely done investigations and clinical examinations. So FGF 23 level monitoring cannot be recommended as a routine prognostic indicator for mortality in critically ill and further trials are needed to evaluate its further applications.

SUMMARY

The present study was carried out at BLDEU B.M PATIL MEDICAL COLLEGE HOSPITAL VIJAYAPUR over a period of 18 months during From December 2018 to May 2020 among the critically ill patients, admitted in the Intensive Care Unit who needed ICU admission for 24 hours or more. Demographic characteristics were noted. Detailed history was noted and clinical examination was done. Serum FGF23 and APACHE scores were documented on day one. Number of days of mechanical ventilation, duration of ICU stays, and duration of hospital stay was also noted. The results showed that FGF 23 level measurement on the day of admission to critical care unit, can predict the outcome of the patient in the form of discharge from hospital or death in the hospital. The survivors also showed lesser FGF 23 values and APACHE scores than non-survivors. Patients who have raised FGF 23 level at admission are more likely to die. Patients who had comorbidities had a poorer prognosis in terms of increased mortality. The duration of mechanical ventilation, duration of ICU stay was not significantly different in either group. However, survivors had a longer hospital stay. From the above study, serum FGF 23 level appears to be one of the indicators predicting the outcome of critically ill patients requiring mechanical ventilation. Its value as an important prognostic marker in CKD is well established and as an independent risk factor it is being studied. It is not a routinely done or widely known test. It can be expensive and may not be available in all laboratories yet in India. It needs more studies to evaluate its efficacy in Indian set up. APACHE II appears to be a superior and simpler score to predict mortality in critical care units.

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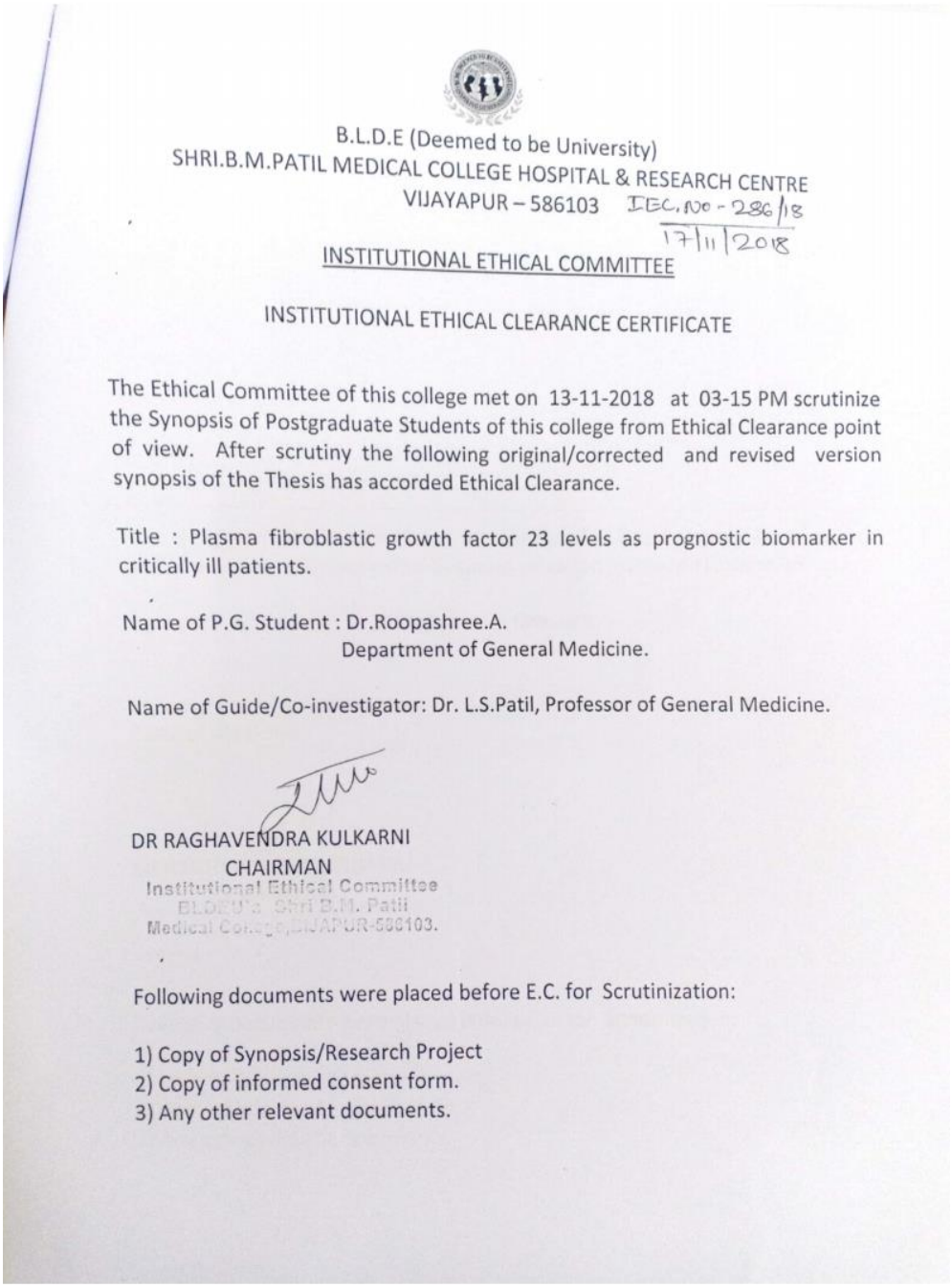
ANNEXURES

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE



B.L.D.E (Deemed to be University)
SHRI.B.M.PATIL MEDICAL COLLEGE HOSPITAL & RESEARCH CENTRE
VIJAYAPUR – 586103 IEC.No - 286/18
17/11/2018

INSTITUTIONAL ETHICAL COMMITTEE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 13-11-2018 at 03-15 PM scrutinize the Synopsis of Postgraduate Students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected and revised version synopsis of the Thesis has accorded Ethical Clearance.

Title : Plasma fibroblastic growth factor 23 levels as prognostic biomarker in critically ill patients.

Name of P.G. Student : Dr.Roopashree.A.
Department of General Medicine.

Name of Guide/Co-investigator: Dr. L.S.Patil, Professor of General Medicine.

DR RAGHAVENDRA KULKARNI
CHAIRMAN
Institutional Ethical Committee
BLDEU's Shri B.M. Patil
Medical College,VIJAPUR-586103.

Following documents were placed before E.C. for Scrutinization:

1) Copy of Synopsis/Research Project
2) Copy of informed consent form.
3) Any other relevant documents.

CONSENT FORM

INFORMED CONSENT FORM **:**"PLASMA FIBROBLASTIC GROWTH FACTOR 23 LEVELS AS PROGNOSTIC BIOMARKER IN CRITICALLY ILL PATIENTS"

GUIDE : DR L.S.PATIL

P.G.STUDENT : DR ROOPASHREE A

PURPOSE OF RESEARCH:

I have been informed that the purpose of this study is to assess the levels of serum FGF23 in critically ill patients.

PROCEDURE:

I understand that I will undergo detailed history and clinical examination and investigations.

RISKS AND DISCOMFORTS:

I understand that there is no risk involved in this study and I may experience mild pain during the above mentioned procedures.

BENEFITS:

I understand that my participation in this study will help to assess the levels of fibroblastic growth factor in critically ill patients in this part of state.

CONFIDENTIALITY:

I understand that the medical information produced by the study will become a part of hospital record and will be subjected to confidentiality and privacy regulation of hospital. If the data is used for publication the identity will not be revealed.

REQUEST FOR MORE INFORMATION :

I understand that I may ask for more information about the study at any time.

REFUSAL OR WITHDRAWAL OF PARTICIPATION :

I understand that my participation is voluntary and I may refuse to participate or withdraw from study at any time.

INJURY STATEMENT :

I understand in the unlikely event of injury to me during the study I will get medical treatment but no further medical compensation

Signature of guardian	Signature of the patient

STUDY SUBJECT CONSENT FORM:

I confirm that DR.ROOPASHREE A has explained to me the purpose of this research, the study procedure that I will undergo and the possible discomforts and benefits that I may experience, in my own language.

I have been explained all above in detail in my own language and I understand the same. I agree to give my consent to participate as a subject in this research project.

Signature of the participant/ date Signature of the witness/date

SCHEME OF CASE TAKING

Name: CASE NO:

Age: OP/IP NO:

Sex: DOA:

Religion: DOD:

Occupation:

Address:

Presenting complaints with duration:

History of presenting complaints:

Past History:

Family History:

Personal History:

Diet

Appetite

Sleep

Bladder and bowel habits:

Others

Treatment History: treatment for diabetes/hypertension

General Physical Examination

Pallor: Present/absent

Icterus: Present/absent

Cyanosis: Present/absent

Clubbing: present/absent

Generalized lymphadenopathy: Present/absent

Odema: Present/absent

Built:

Nourishment:

Vitals

PR:

BP: in mm of mercury (mm hg)

RR:

Temp:

SYSTEMIC EXAMINATION.

- Cardiovascular system
- Respiratory system
- Per abdomen
- Central nervous system

INVESTIGATIONS

PATHOLOGY

1.) Complete blood count:

Hb	gm/dl
Total count	Cells/cumm
Differential count	
Neutrophils	%
Lymphocytes	%
Eosinophils	%
Basophils	%
Monocytes	%

1.) ESR

2.) Urine Routine

BIOCHEMISTRY

- Serum magnesium

- FGF 23
- Random blood sugar
- Fasting blood sugar
- Post prandial blood sugar
- Liver function test
- Renal function test
- ABG

RADIOLOGY

- Chest X ray
- USG Abdomen
- CT Brain

Other relevant investigations will be done when required.

APACHE II SCORE

Acute Physiology score

- Rectal temperature
- Mean Blood pressure
- Heart rate
- Respiratory rate
- Arterial PH
- Oxygenation
- Serum sodium
- Serum potassium
- Serum creatine

- Hematocrit
- WBC count

Glassgow Coma Scale

- Eye Opening
- Verbal
- Motor activity

Points Assigned to age and Chronic disease

Chronic health Diseases

CONCLUSION:

DATE: **SIGNATURE:**

Apache II score table

Physiologic Variable		+4	+3	+2	+1	0	+1	+2	+3	+4
Temperature - rectal (°C)		≥41	39-40.9		38.5-38.9	36-38.4	34-35.9	32-33.9	30-31.9	≤29.9
Mean Arterial Pressure (mm Hg)		≥160	130-159	110-129		70-109		50-69		≤49
Heart Rate		≥180	140-179	110-139		70-109		55-69	40-54	≤39
Respiratory Rate (nonventilated or ventilated)		≥50	35-49		25-34	12-24	10-11	6-9		≤5
Oxygenation (mmHg) a. $FiO_2 \geq 0.5$ use A-aDO_2 b. $FiO_2 < 0.5$ use PaO_2	a.	≥500	350-499	200-349		<200				
	b.					> 70	61-70		55-60	<55
Arterial pH		≥7.7	7.6-7.69		7.5-7.59	7.33-7.49		7.25-7.32	7.15-7.24	<7.15
Serum Sodium (mmol/L)		≥180	160-179	155-159	150-154	130-149		120-129	111-119	≤110
Serum Potassium (mmol/L)		≥7	6-6.9		5.5-5.9	3.5-5.4	3-3.4	2.5-2.9		<2.5
Serum Creatinine (mg/dL, Double point score for acute renal failure)		≥3.5	2-3.4	1.5-1.9		0.6-1.4		<0.6		
Hematocrit (%)		≥60		50-59.9	46-49.9	30-45.9		20-29.9		<20
White Blood Count (in 1000/mm³)		≥40		20-39.9	15-19.9	3-14.9		1-2.9		<1
Glasgow-Coma-Scale (GCS)		Score = 15 minus actual GCS								
Serum HCO_3 (venous, mmol/L, use if no ABGs)		≥52	41-51.9		32-40.9	22-31.9		18-21.9	15-17.9	<15
A = Total Acute Physiology Score APS		Sum of the 12 individual variable points								

B – Age Points	
≤44 years	0 points
45-54 years	2 points
55-64 years	3 points
65-74 years	5 points
≥75 years	6 points

C – Chronic Health Points

If the patient has a history of severe organ system insufficiency or is immunocompromised assign points as follows:

a. For nonoperative or emergency postoperative patients – 5 points
b. For elective postoperative patients – 2 points

APACHE II Score = Sum of A (APS points) + B (Age points) + C (Chronic Health points)

KEY TO MASTER CHART

NAME

AGE IN YEARS

SEX-

MALE FEMALE

CAP	Community Acquired Pneumonia
COPD	Chronic Obstructive Pulmonary Disease
CVA	Cerebro Vascular Accident
DKA	Diabetic Keto Acidosis
GBS	Guillian Bare Syndrome
GI Bleeding	Gastro-Intestinal Bleeding
TBI	Traumatic Brain Injury
OPC	Organo-Phosphorous Compound
LVF	Left Ventricular Failure
DM	Diabetes Mellitus
HTN	Hypertension
IHD	Ischaemic Heart Disease
GCS	Glasgow Coma Scale
Rf	respiratory failure
Y	yes
N	no

MASTER CHART

s.no	age	sex	Height	weight	BMI	Diagnosis	DM	HTN	ihd	Comorbidities	Reason for intubation	APACHE II	FGF23	days on ventilation	days in icu	days in hospital	outcome
1	67	female	156	60	24.7	cva	yes	yes		y	poor gcs	29	268.1996	14	14	14	non-survivor
2	51	female	164	61	22.7	OPC	yes			y	rf	26	264.8629	12	12	12	non-survivor
3	32	female	154	62	26.1	cap				n	rf	27	536.647	7	7	7	non-survivor
4	66	female	158	63	25.2	lvf	yes		yes	y	rf	28	268.7576	12	12	12	non-survivor
5	52	male	153	64	27.3	septic shock	yes			y	severe shock	22	353.2238	7	9	12	survivor
6	59	male	172	65	22.0	COPD	yes	yes	yes	y	rf	34	358.6897	12	16	22	survivor
7	40	male	173	66	22.1	cap	yes			y	rf	35	204.8178	6	6	6	non-survivor
8	70	male	172	67	22.6	lvf			yes	y	rf	32	253.0926	7	7	7	non-survivor
9	39	male	171	68	23.3	septic shock	yes	yes		y	severe shock	33	262.0198	3	3	3	non-survivor
10	34	male	178	69	21.8	cap	yes			y	rf	29	273.2631	12	12	12	non-survivor
11	37	female	162	69	26.3	cva		yes		y	poor gcs	37	541.2543	11	11	11	non-survivor
12	58	female	153	70	29.9	septic shock		yes		y	severe shock	33	365.5241	4	4	4	non-survivor
13	52	female	154	71	29.9	septic shock	yes			y	severe shock	27	375.5302	5	9	14	survivor

14	68	female	155	72	30.0	lvf		yes	yes	y	rf	29	375.6191	4	4	4	non-survivor
15	65	female	156	73	30.0	COPD	yes	yes		y	rf	30	383.6488	6	6	6	non-survivor
16	59	female	157	74	30.0	dka	yes	yes		y	severe acidosis	34	386.5768	8	8	8	non-survivor
17	51	female	158	75	30.0	Meningitis				n	poor gcs	26	388.9751	14	14	14	non-survivor
18	49	female	159	76	30.1	cva		yes		y	poor gcs	24	276.4698	7	8	9	survivor
19	46	male	180	80	24.7	cap	yes	yes		y	rf	21	448.8785	8	9	14	survivor
20	57	male	181	81	24.7	OPC				y	rf	32	485.0971	14	14	14	non-survivor
21	60	male	175	82	26.8	septic shock	yes			y	severe acidosis	35	495.2442	3	3	3	non-survivor
22	60	male	175	83	27.1	hyponatremia	yes	yes	yes	y	poor gcs	30	218.3802	3	5	6	survivor
23	60	female	167	85	30.5	cva	yes	yes		y	poor gcs	30	274.8643	8	9	10	survivor
24	56	female	167	86	30.8	septic shock	yes		yes	y	severe shock	29	330.3917	6	7	8	survivor
25	46	female	179	87	27.2	cap	yes			y	rf	21	232.9115	8	10	16	survivor
26	43	female	167	87	31.2	hanging	yes	yes		y	poor gcs	32	333.3805	14	15	14	survivor
27	47	female	161	87	33.6	cap	yes			y	rf	22	414.8886	9	11	14	survivor
28	52	male	180	88	27.2	GB syndrome				n	rf	22	242.7125	6	7	9	survivor

29	58	female	161	88	33.9	septic shock		yes		y	rf	33	427.3501	6	6	6	non-survivor
30	66	female	164	90	33.5	cap			yes	y	rf	31	407.0619	26	26	26	non-survivor
31	53	male	167	92	33.0	lvf	yes	yes		y	rf	28	585.2012	4	4	4	non-survivor
32	50	female	174	93	30.7	COPD		yes		y	rf	25	327.4357	4	6	8	survivor

33	70	male	168	93	33.0	septic shock			yes	y	severe	36	545.45	4	4	4	non-survivor
34	49	male	172	97	32.8	hanging	yes			y	poor gcs	24	350.1234	12	12	12	non-survivor
35	68	male	173	98	32.7	lvf	yes	yes	yes	y	rf	29	346.5821	5	5	5	non-survivor
36	37	male	174	99	32.7	cva		yes		y	poor gcs	32	343.918	6	6	6	non-survivor
37	65	male	175	100	32.7	cva	yes	yes	yes	y	poor gcs	30	341.0426	8	8	8	non-survivor
38	60	male	176	101	32.6	tbi	yes	yes		y	poor gcs	35	191.7102	10	10	10	non-survivor
39	34	male	177	102	32.6	cap				n	rf	26	337.8517	13	15	17	survivor
40	48	male	178	103	32.5	myasthenia gravis				n	rf	18	333.8459	5	7	9	survivor
41	26	female	164	59	21.9	tbi				n	poor gcs	21	299.7436	10	11	14	survivor
42	49	female	162	68	25.9	OPC				n	rf	19	302.2585	8	9	11	survivor
43	49	female	162	70	26.7	cap				n	rf	24	302.7075	6	7	10	survivor

44	54	female	171	71	24.3	cva		yes		y	poor gcs	24	148.6411	8	9	12	survivor
45	60	male	172	72	24.3	tbi			yes	y	poor gcs	30	326.9182	8	10	13	survivor
46	56	male	173	73	24.4	OPC	yes			y	rf	26	350.4337	9	10	12	survivor
47	49	male	174	74	24.4	Meningitis				n	poor gcs	19	358.1111	4	5	8	survivor
48	35	male	175	75	24.5	gi bleed				n	airway protection	26	364.9647	4	5	6	survivor
49	48	male	176	76	24.5	cva		yes		y	poor gcs	23	365.2477	14	18	22	survivor
50	35	male	177	77	24.6	OPC				n	rf	22	396.0368	5	10	12	survivor
51	52	female	160	77	30.1	tbi		yes		y	poor gcs	22	303.2585	7	9	12	survivor
52	51	male	178	78	24.6	OPC		yes		y	rf	26	274.9305	7	7	7	non-survivor
53	55	male	179	79	24.7	cva		yes	yes	y	poor gcs	25	275.6342	6	7	9	survivor
54	51	male	175	84	27.4	OPC		yes		y	rf	21	278.0113	10	11	12	survivor
55	22	female	161	86	33.2	GB syndrome				n	rf	24	304.7792	8	9	11	survivor
56	43	male	177	88	28.1	tbi			yes	y	poor gcs	18	283.2167	16	16	16	non-survivor
57	47	male	181	89	27.2	cap		yes		y	rf	22	275.6832	8	9	11	survivor
58	46	male	177	89	28.4	cap		yes		y	rf	21	283.9337	7	9	11	survivor

59	60	female	161	89	34.3	cva	yes	yes		y	poor gcs	30	306.6085	5	7	9	survivor
60	35	male	182	90	27.2	tbi				n	poor gcs	29	340.0833	6	8	12	survivor
61	59	male	177	90	28.7	cva		yes		y	poor gcs	29	284.1389	8	9	11	survivor
62	60	male	166	91	33.0	lvf	yes	yes		y	rf	30	295.711	5	8	11	survivor
63	76	male	160	91	35.5	snake bite			yes	y	rf	19	296.7991	6	8	12	survivor
64	63	female	158	91	36.5	OPC	yes	yes		y	rf	33	308.9717	4	7	10	survivor
65	38	male	174	92	30.4	tbi		yes		y	poor gcs	26	290.5717	9	11	12	survivor

66	51	female	158	92	36.9	Meningitis		yes		y	seizures	21	319.652	5	6	8	survivor
67	52	female	158	93	37.3	Meningitis	yes			y	rf	22	322.6414	4	6	8	survivor
68	55	male	169	94	32.9	cap			yes	y	rf	30	294.1323	7	8	9	survivor
69	61	female	169	94	32.9	lvf		yes	yes	y	rf	31	304.5239	6	8	10	survivor
70	56	male	170	95	32.9	cva	yes			y	poor gcs	26	293.27	10	11	13	survivor
71	66	female	162	95	36.2	lvf	yes	yes	yes	y	rf	36	308.248	6	7	9	survivor
72	41	male	171	96	32.8	cap				n	rf	16	292.409	12	13	15	survivor