.....MATERIALS AND METHODS

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The study was carried out in 144 patients including those considered as controls, from two different hospitals in Baroda - Shri Sayaji General Hospital (SSGH) and Bhailal Amin General Hospital (BAGH). Eighty patients studied had suffered acute biological stress in form of either sever trauma causing fractures of at least two long bones, (head injuries were excluded) or had undergone major surgery. Twenty-four patients of BAGH ICU were suffering from serious diseases causing functional failure of at least two or more major organs of the body. The control group comprised of 20 patients each from SSGH and BAGH who belonged to surgical, medical and orthopedic wards suffering from minor aliments and not requiring surgical intervention. Patients' general information like nature of trauma, blood pressure, weight, nature of treatment, presence of other associated diseases like diabetes, liver disorder, kidney failure, hypertension etc. were also inquired (Appendix 1 to 5). Special attention was given to mode of nutrition (enteral / parenteral), approximate caloric intake by the patients.

All patients were subjected to biochemical tests like blood sugar, serum insulin, serum albumin, serum transferrin, serum creatinine and blood urea in fasting state. Care was taken to hold back the intravenous nutrition for 15

minutes before collecting the blood sample. Blood tests like albumin and transferrin were repeated on 4^{th} and 8^{th} day in order to asses the serial change in protein levels. In patients with multiorgan failure some additional tests were noted from hospital records like Arterial blood gas analysis, sodium (Na^+) and potassium (K^+) concentrations.

METHOD FOR COLLECTION OF BLOOD

Time: Early morning in fasting state and/or when the ongoing intravenous drip was held for 15 minutes before blood collection.

Collection: 2cc in sodium fluoride bulb for blood sugar. 5cc in plain bulb for serum analysis which was centrifuged after half an hour of blood collection and then serum was separated and stored at –20°C temperature till needed.

BIOCHEMICAL ANALYSIS

SERUM ALBUMIN: Spectroscopic method using Bromocresol Green (BCG) was employed to estimate serum albumin. (Autopak® Reagent Kit M/s Miles India Ltd.)

Reagents: Bromocresol green (BCG), Standard Albumin (5g/dL).

Procedure: The samples and reagents were brought to room temperature prior to use and pitted as follows

	Standard	Blank	Test
BCG	2.0ml	2.oml	2.0ml
Serum (Sample)			0.02ml
Distilled Water		0.02ml	
Standard	0.02ml		

The sample tubes were incubated for 1 minute and read at 630 nm.

SERUM TRANSFERRIN: Serum Transferrin was assayed by immunoturbidimetric method using Autopak® reagent kit of M/s Miles India Ltd.

Reagents: Antibody reagent, PEG solution, Diluent calibrator.

Procedure: The samples and reagents were brought to room temperature prior to use and pitted as follows

	Blank	Calibrators	Sample
Diluent	20µl		
Working calibrators		20µl	
Prediluted sample			20µl
Antibody reagent	1.0ml	1.0ml	1.0ml

Mixed well and allowed to stand at room temperature for 20 minutes. Read the absorbance of working calibrators and sample against the blank at 340 nm.

SERUM UREA: Serum urea was assayed by the method of Talke and Schubert (1965) using reagent kit of M/s Preccugent.

Reagents: α-Ketoglutarate, NADH, Urease, GLDH, ADP, Tris buffer

Procedure: The samples and reagents were pipetted into cuvettes as follows

	Standard	Unknown
Reagents	1.0ml	1.0ml

Brought to 37°C, Zero Spectrophotometer with blank

Standard	0.01ml	
Specimen		0.01ml

Mix gently. Record initial absorbance (A1) 60 second after mixing and final absorbance (A2) 120 seconds after mixing at 340 nm.

SERUM CREATININE: Serum creatinine was estimated using creatinine kinase reagent procured from M/s Preccugent.

Reagents: Creatine phosphate, ADP, NAD, NAC, Hexokinase, G-6PDH, D-Glucose, Magnesium ions, EDTA, Buffer6.1 pH

Procedure: The samples and reagents were pipetted into labeled tubes as follows;

Reagent	1.0ml

Brought to 37°C, Zero Spectrophotometer with blank

Specimen	25µl

Mixed and incubated at 37°C. Take first absorbance at 120 sec after mixing and second and third absorbance reading at 180 seconds and 240 seconds respectively after mixing.

PLASMA GLUCOSE: GOD/POD method was used for the quantitative determination of glucose. Autopak® reagent kit supplied by M.s Bayer Diagnostics India Ltd, was used.

Reagents: Buffer / enzyme (GOD/POD)/Chromogen (4-Aminophenazone, Phenolic Compound) , Standard: Glucose

Prepration of working solution

Gentely dissolved 1 tablet in 20 ml of distilled water in clean beaker, with continuous stirring. Transfered the solution in a dark bottle which is considered as Working solution, ready to use.

Procedure: Sample and reagents were pipetted as follows

	Standard	Blank	Test
Working Solution	2.0ml	2.0ml	2.0ml
Sample			2.0ml
Distil Water		2.0ml	
Standard	2.0ml		

The tubes are incubated for 15 minutes and read at 520nm.

PLASMA INSULIN: Plasma insulin is assayed by Enzyme-immunological test for the quantitative determination of human insulin in vitro by Boehringer Mannheim Immunodiagnostics kit by ELISA.

Reagents: Phosphate buffer, Anti-insulin-POD conjugate, Standards insulin in bovine serum matrix, Substrate/buffer (phosphate/citrate),Chromogen.

Procedure: Sample and reagents were pipetted as ,

	Standard a-e	Control Serum	Sample
	0.1 ml	0.1 ml	0.1 ml
Solution 1 a	1.0 ml	1.0 ml	1.0 ml

The tubes were incubated for 120 minutes and then contents were aspirated, discarded and tubes were rinsed by filling to the brim with Enzyme-test Washing solution immediately. This solutions were completely aspirated within 3-15 minutes. Again pipetted as,

	Standard a-e	Control Serum	Sample
Substrate chromogen Solution	1.0 ml	1.0 ml	1.0 ml

The tubes were incubated for 60 minutes and then the contents were mixed well and read at 420 nm.

ARTERIAL BLOOD GAS ANALYSIS: Analysis was done in an Blood Gas Analyzer (CIBA-CORNING,278, Blood Gas System) based on principal of ION SPECIFIC/SELECTIVE ELECTRODE (ISE), in BAGH laboratory, using heparinized arterial blood.

STATICAL ANALYSIS The results are expressed as mean ± standard error. The data were subjected to analysis of variance (ANOVA) followed by Duncan's Multiple Comparison