ISSN No:-2456-2165

A Review on Enzymes as Diagnostic Reagents and Therapeutic Agents

Afreen H[1], Kiran Manoj[2], Rajeev Nateshkumar[3]
Anna University Chennai[1], Central University of Rajasthan[2], University of Mumbai[3]

Abstracts:- The present review is based on papers published between 1972 and 2020 and gives Comparative information about the most common enzymes as Diagnostic reagents and their clinical applications in disorders. Activities of following enzymes: Acid Phosphatase, Alanine Aminotransferase, Alkaline Phosphatase, Cholinesterase, Creatinine Kinase, Gamma Glutamyl Transferase, Lactate Dehydrogenase, Renin, Urokinase.

Keywords:- *Enzymes, Renin, Therapeutic applications of enzymes, Diagnostic Reagents, Phosphatase.*

I. INTRODUCTION AND DISCUSSION

❖ *Cholinesterase*

➢ *Quantitative analysis and estimation of cholinesterase for diagnostic purposes for laboratory and commercial use.*

The different quantification methods being employed for the estimation of cholinesterase have been discussed briefly. There is an increasing need for reliable tests for assessing the cholinesterase status of patients. This will be useful in identifying the poisonings caused by cholinesterase inhibitors such as the organophosphate or carbamate insecticides.

The basic hydrolysis of acetylcholine, a neurotransmitter, yields choline and acetic acid, of which the acetic acid produced needs to be quantified to measure the cholinesterase activity in a specimen. The different methods that have been studied and utilised for clinical and commercial purposes are briefly stated as follows: electrometric, titrimetric, manometric and colorimetric methods. The main criteria for the assays were sensitivity and reproducibility.

The titrimetric method utilised the pH-stat where electronic means are used to maintain a constant pH. This method is very sensitive and the most reproducible but the only limitation was that the cost of the equipment is high. The manometric method is also sensitive but it is more time consuming in comparison with the other methods. Graded colour changes are observed under controlled reaction conditions in the colorimetric method and this method could be employed in places with no access to laboratories as well.

There originates a confusion in interpreting the results as there are several modes/units to express cholinesterase activity and thus a careful standardization of the different units needs to be done.

The main applications of these methods could be helpful in identifying the serum cholinesterase levels which may be an indication of some disease or disorder, especially of the nervous system as acetylcholine is present in the synaptic junctions of the neurons and functions as a chemical messenger. This could further help to develop commercial reagent kits or point of care devices for the accurate and early diagnosis of the said disorders.

➢ *Activity of phenyl carbamate analogues of Rivastigmine as a novel therapeutic agent for the treatment of Alzheimer's disease.*

There have been several strategies that have been followed for the treatment of Alzheimer's of which the use of cholinesterase inhibitors is the most studied and most used form of palliative treatment. Due to many advantageous features, donepezil has been the most used drug.

In this paper, the studies have been centred around rivastigmine which is the next best treatment and the pros and cons have been elaborated. Rivastigmine shows an interesting selectivity in the brain regions that are most affected by neuronal degradation. Some limitations in the usage of this drug is the short half-life period, difficulty in intake and the high cost associated with the synthesis of its intermediate – N-ethyl-N-methyl carbamoyl chloride.

Some recent studies have been conducted on dimethyl, diethyl and n-hexyl carbamates related to rivastigmine to try and overcome these limitations. The synthesised compounds have been evaluated for the acetylcholinesterase and butyrylcholinesterase in different cerebral regions such as the cortex, striatum and hippocampus. The LD50, IC50 and ED50 values have been estimated for the several compounds.

Thus, the therapeutic effects of the carbamate derivatives of Rivastigmine have been analysed for their efficacy and toxicity. Conformational analysis has also been performed to determine the minimal energy conformations that is best suited for our needs. The different compounds have been compared and analysed to suggest which of them would be

useful as a potential drug candidate. The further clinical trials have not been done on this drug and there is still much more information to be understood before it could be decisively considered as a potential candidate.

➢ *Clinical evidence-based studies recommendations for the use of cholinesterase inhibitors – A Literature Review*

Cholinesterase inhibitors have been a standard therapy for patients with Alzheimer's disease. Tacrine was the first used compound for the treatment of AD, but it was later replaced by donepezil due to the substantial hepatotoxicity it caused. Donepezil (Aricept), rivastigmine (Exelon) and galantamine (Reminyl) have been considered for this study.

The papers that have been collected for this review were grouped into 3 classes; Class I that included randomised, controlled, clinical trials and overviews (meta-analysis); Class II that included well designed observational studies with concurrent controls and Class III that included expert opinions, case series, case reports and historical controls.

The 3 cholinesterase inhibitors that have been considered in this study have different half-life and metabolisms. Donepezil has a longer half-life in comparison with the other two drugs. Donepezil and galantamine are metabolised with the help of cytochrome p450 enzymes while rivastigmine is hydrolysed to a phenolic by product and excreted through urine. Donepezil has the highest protein binding. Galantamine and rivastigmine have delayed absorption caused by intake of food.

MEDLINE and PUBMED are scientific journals that have been used as a source for the papers that have been published and a comparison in action between the different drugs employed and a spectrum of varying responses have been identified from patients and the outcomes have been distinguished as primary and secondary outcomes.

The overall efficacy of these cholinesterase inhibitors has been thoroughly summarised based on a comparative study of all the literature that has been published. This helps us understand the precise action of the drug, the possible side effects and also if it gives rise to any comorbidities depending on the patient's medical history. However, the clinical issues of when to stop the treatment have not been studied or reported. There is an absence of direct comparative trials.

➢ *Multi-target directed ligands against cholinesterase for Alzheimer's treatment based on modifications on Donepezil*

Despite the detailed studies on Alzheimer's, only very few drugs have been clinically approved by the Food and Drug Administration (FDA). Donepezil, one of the four drugs that have been approved has been discussed in connection with the development of multi-target directed ligands (MTDLs).

Donepezil, is a non-competitive, reversible cholinesterase inhibitor with high selectivity used to reduce neuronal degradation. The limitation is that it is not able to penetrate the blood brain barrier (BBB), thereby exhibiting a limited potential in treatment of AD.

The different hypotheses regarding the pathogenesis of Alzheimer's are discussed here including the cholinergic hypothesis, amyloid hypothesis, oxidative stress hypothesis and bio metal hypothesis. It has been discussed how different structural/biochemical modifications have been done on the donepezil and its analogues and how effective they are in the treatment of Alzheimer's.

With the help of molecular docking, several different compounds have been produced by making structural modifications to the enzyme, and this could serve as potential drug targets. This is the main idea behind the development of multi-target directed ligands (MTDLs). The main properties that are being focussed are the BBB permeation, additional antioxidant property, additional inhibition, ROS scavenge ability, improving memory performance, etc.

Of the available drugs, almost all of them are only used to relieve the symptoms or slow down the progression of the disease. Hence, it is the need of the hour to develop hybrids of the available drugs with a MTDL property wherein two or more distinct pharmacophores that recognise diverse targets that may be closely related to AD are combined to yield a drug with multiple benefits and minimal side effects. If more detailed analysis is done, some of these compounds could be shortlisted to be potential drug targets for the treatment of Alzheimer's.

❖ *Creatine Kinase*

➢ *Serum creatine kinase as a biomarker in systemic lupus erythematosus.*

This paper summarises a case report of a patient prognosis and treatment administered for systemic lupus erythematosus (SLE). The different therapies that have been administered and the side effects caused have been studied. A better understanding of the primary SLE-induced infection of the musculoskeletal system and the drug-induced side effects is important for appropriate treatment of SLE patients.

The patient had initially presented with arthralgia, arthritis, myalgia, pleuritis, and Raynaud's phenomenon and was diagnosed as having a mixed connective tissue disease. Glucocorticosteriods and chloroquine were provided as a treatment. Later, autoantibodies to double-stranded DNA were detected. The elevated creatine kinase levels have shown to indicate musculoskeletal involvement in SLE.

The treatments were switched between cyclophosphamide, azathioprine and chloroquine as due to noncompliance. The primary aim of this case study was to

understand the different problems that arose due to the development of SLE and how the creatine kinase levels in the serum is used as indicators for the malfunctioning of different tissues.

Different drugs have been administered as a palliative treatment for the symptoms, the usage is withdrawn when it gives rise to more severe side effects and later re-introduced if needed. Thus, it is essential for us to understand the patient's medical history better and try to develop potential drug targets that could be of a multi-target drug ligand, to reduce the use of multiple drugs and well as specifically designed to improve the efficacy and reduce the toxicity.

- *Creatine kinase, phosphate and lipid values as biomarkers in patients with chronic myeloid leukaemia and their response to Imatinib treatment.*

Chronic myeloid leukaemia (CML) is a blood cell cancer that begins in the bone marrow whose cause may be due to the occurrence of a spontaneous mutation. Imatinib has been widely used as a treatment for CML but some abnormalities have been identified during the therapy. This paper summarises the differences in some biomarkers' values that is indicative of the presence of some abnormalities or comorbidities.

There are some significant side effects caused due to treatment with imatinib. Some of the abnormalities that are observed during imatinib treatment are – increased creatine kinase levels, inverse creatine kinase-phosphate correlation, differences in the cholesterol and triglyceride values and overall decrease in phosphate. Creatine kinase is an enzyme that catalyses the formation of phosphocreatine with the help of ATP, which in turn is an energy reservoir and a part of the intracellular energy transport.

Diarrhoea may lead to malabsorption, which further leads to decreased serum calcium levels resulting in secondary hyperparathyroidism. This in turn results in an increased excretion of phosphorus which is one of the abnormalities. The platelet derived growth factor is inhibited by imatinib which leads to a decrease in the lipoprotein lipase synthesis which ultimately results in a decrease in a decrease in LDL cholesterol.

The creatine kinase, phosphate and triglyceride levels over a period of 3 years have been measured at a 6-month interval in patients receiving the imatinib treatment. Although CK is an enzyme that is primarily associated with the musculoskeletal system, there has been no correlation found between muscle cramps and CK levels in a patient undergoing this therapy.

CK is assayed in blood tests as a marker of damage of the CK rich tissues, such as, skeletal muscle, brain, photoreceptor of retina, hair cells of inner ear, spermatozoa and smooth muscle cells. The overall aim of the study is to contribute data that could help define abnormalities related to imatinib therapy for easier clinical recognition.

- *A study of Etoposide treatment of breast cancer using Ehrlich solid tumour induced cardiac toxicity in female mice.*

Breast cancer is the most widely present among women and Ehrlich solid tumours (EST) are used as experimental models for breast cancer. The basis of treatment for cancer involves the activation of apoptosis by the chemical or physical damage of DNA. In this paper, the efficacy and toxicity of etoposide has been studied in EST induced female mice.

The significant features of an Ehrlich carcinoma are that it is an undifferentiated carcinoma with rapid proliferation, has no regression, high translatable capability with 100 percent malignancy, no tumour specific transplantation antigen with a short life span. Etoposide is a chemotherapeutic drug extracted from the plant, Podophyllum pellatum and inhibits the activity of the topoisomerase II enzyme. It is also known to be a semisynthetic derivative of podophyllotoxin.

The experiments were performed on 40 female Swiss albino mice weighing 20–25 g and they were randomized and housed under ambient room- temperature and relative humidity conditions. EST has been induced in these mice that have been divided into four groups based on different doses of drug injected and the means of administration.

The sampling of blood drawn from the different mice over different time intervals showed several changes in the cardiac functions, enzymes and electrolytes. The cardiac function biomarkers are serum lactate dehydrogenase (LDH) and myoglobin creatine kinase (CK-MB) while the electrolyte biomarkers are the serum levels of K+, Na+, Ca2+ and Cl2-.

The main aim of the paper seems to focus on the therapeutic activity of Etoposide and its effectiveness in the treatment of EST which could be extrapolated to study breast cancer studies. The major limitation of this study is that it has been done on animal models and there is no data about human trials. Also, the possible side effects that may be induced on its application to humans has not been discussed.

- *Short-term and long-term treatment using chloroquine and hydroxychloroquine and the associated toxicities.*

The efficacy and the safety involved in the short term, long term and overdose of these antimalarials such as chloroquine and hydroxychloroquine has been discussed and the possibility of them being used as a potential treatment for connective tissue disorders like systemic lupus erythematosus (SLE) and rheumatoid arthritis. It has also been recommended for use recently against COVID-19 but due to some complications caused it has been withdrawn from use.

Chloroquine, hydroxychloroquine and derivatives of 4-aminoquinolone have been used as a treatment against malaria for a long time of which the latter two have a better safety profile. Case reports have described neuromyotoxicity presenting as myositis and muscle weakness with and without elevated creatine kinase activities.

The short-term use is mainly aimed at the prevention and treatment of mild cases. The toxicities induced by the use of these drugs affect different parts of the body, for example, gastrointestinal, dermatologic, neuropsychiatric, glucose abnormalities, cardiotoxicity and many others. A notable toxicity relevant to our field of interest is an elevation of creatine kinase and creatine kinase-MB when higher doses of chloroquine was administered as a treatment for COVID-19.

This paper highlights the different toxicities that have been associated with the short term and long-term use of chloroquine and hydroxychloroquine as a treatment for several diseases including the recently prevalent global pandemic – COVID-19.

The significant drug–drug interactions of the several drugs administered as a treatment for many other diseases with chloroquine and hydroxychloroquine have been summarised. Drug interactions may increase the risk of toxicity occurring. There is a need for more extensive research regarding the toxicities and concentration of drugs administered to give constructive advice regarding these drugs and a complete study of the patient's medical history needs to be done to avoid any comorbidities that may arise as a side effect.

❖ *Gamma Glutamyl transferase*

➢ *The incidence of non-alcoholic fatty liver disease and current methods for diagnosis and treatment.*

Non-alcoholic fatty liver disease (NAFLD) is characterised by hepatic steatosis with varying degrees of inflammation and fibrosis. If left untreated it could progress to form cirrhosis of the liver, liver failure and cancer. This paper talks about the significance of the gamma glutamyl transferase (GGT) enzyme which is a prominent diagnostic biomarker for liver disease. Elevated levels of GGT in the serum is an indication of the presence of disease in the liver, biliary system or pancreas.

The notable pathogenesis associated with NAFLD are genetic polymorphisms, unhealthy dietary patterns, lack of physical activity, insulin resistance, dysbiosis of gut microbiota and some endocrine abnormalities. Some conditions that are co-occurring with NAFLD are systemic hypertension, dyslipidaemia, hypothyroidism, obstructive sleep apnoea, hypopituitarism, etc. The gold standard for diagnosis available currently is liver biopsy which is very effective in determining the severity of the disease and also in staging the liver injury. On the other hand, there are several limitations to this approach, such as, invasiveness of the procedure, cost limitation, sampling errors, occasional morbidity and mortality and this has hence given rise to the need for non-invasive and more precise methods.

The laboratory tests performed include the detection of serum aminotransferases, gamma glutamyl transpeptidase and ferritin whose levels are abnormal in NAFLD.

The occurrence of NAFLD in the recent years has been increasing exponentially and there are still not many proper established studies about the pathogenesis of the disease. Due to the co-occurrence of several conditions there is a need for better and safe diagnosis. Gamma glutamyl transferase enzyme functions merely as an indicator of liver damage but unfortunately it is not highly specific and could indicate the presence of some other disease as well.

➢ *Kidney and liver related disorders and their correlation with multimorbidity patterns and polypharmacy in the older age group.*

This paper summarises the correlation between multimorbidity, which is the co-occurrence of multiple chronic medical conditions, and polypharmacy, which is the concomitant consumption of five or more drugs, and their effects on the diseases associated with the kidneys and liver in old people. This has been done to analyse the medication that is in use and to understand the side effects that may likely be caused.

The several methods that have been employed in this study include designing, settling and studying the population, data sources and variables. Some of the variables taken under consideration are chronic diseases and their multimorbidity, death and dropout count, drugs and polypharmacy, kidney, liver functions and some other variables. Alkaline phosphatase, gamma glutamyl transferase and alanine transaminase serve as crucial biomarkers in the detection of a diseased hepatic system.

The drugs have been divided into several different clusters according to the disease they are used as a treatment for and it has been tried to determine whether they cause any abnormalities in the liver or kidney.

The most over represented drugs in each Cluster coincide with the most over-represented disorders in that same cluster. Patients who tend to have multimorbidity and as a result receive polypharmacy have been identified to present abnormal kidney and liver functions. But unfortunately, the exact cause for this has not yet been identified.

The main aim of this study is to try to address the issues prevalent in the use of several drugs for the treatment of the multiple diseases that may occur in old people. It can be noted that sometimes it is essential to prescribe multiple drugs for the treatment of a disease but at the same time consumption of numerous medications will mostly definitely lead to a reduced

efficacy and possible side effects or comorbidities. Hence it is essential for the prescriptions to be extremely precise and try to limit the number of drugs as much as possible.

- *Oral vancomycin as a therapeutic agent for primary sclerosing cholangitis and the monitoring of gamma glutamyl transferase levels in such patients.*

Primary sclerosing cholangitis (PSC) is a chronic, fibroinflammatory, immune mediated disease of the bile ducts. It could cause hepatobiliary and colorectal malignancy. Till date there has been no proper treatment suggested for this. There seems to be some association of PSC with inflammatory bowel disease and ulcerative colitis.

The 'leaky gut' theory states that the pathogenic gut microbiota tend to cross through the inflamed gut wall into portal circulation and travel down the biliary tree causing PSC. Vancomycin has been suggested as a possible form of treatment.

The studies have been done both on children and in adults. The laboratory parameters include ALT, ALP and GGT and these levels at baseline have been assumed. Oral administration of vancomycin has been proven to show a decrease in the intestinal inflammation of IBD on colonic biopsies. A primary outcome of this study is the decline in the serum levels of the above-mentioned biomarkers, improvements in the biliary structures and dilatation.

GGT is said to be a more accurate and reliable biomarker in children when compared to ALP as the ALP present in the serum is usually the sum of it from the liver and the bones. So, a high level of ALP could also be an indicative of some problem with the bones and it is not entirely specific to this area of interest.

Normalisation of the serum levels of these biomarkers enables a better hepatobiliary related survival in patients with PSC. Some of the limitations of this study are the size of the subgroup, lack of control and placebo groups and the need to have used more narrow time intervals. Also, there is a requirement for more accurate biomarkers, preferably specific to the disease being discussed to enable more effective treatment procedures.

- *The role of GGsTop as a potential inhibitor of γ-GT activity and its effectiveness in controlling ischemia reoxygenation injury in patients with liver related disorders.*

This paper discusses the effect of GGsTop, which is a potent inhibitor of γ-glutamyl transpeptidase (GT) which serves as an indicator for the presence of hepatic steatosis. Hepatic steatosis is accompanied by the presence of ischemia reoxygenation injury, which is the tissue damage caused when blood supply returns to the tissue after a period of ischemia (lack of oxygen). With the increasing incidence of NAFLD, it has become imperative to prevent the hepatic IR injury in patients with the disease.

Non-alcoholic fatty liver disease (NAFLD) is one of the most common chronic liver diseases which has an increased risk of developing diabetes, hypertension, cardiovascular events, abnormal resting electrocardiography and endothelial dysfunction. γ-GT catalyses the initial step in the glutathione (GSH) degradation and transfers the γ-glutamyl moiety of GSH to water and amino acids or peptides (transpeptidation) into glutamate and γ-glutamyl amino acids or peptides.

(2-amino-4{[(3-carboxymethyl) phenyl] (methyl) phosphono} butanoic acid), or GGsTop is a potent inhibitor of γ-GT and it also helps in the reduction of hepatic ischemia-reperfusion injury and ameliorates oxidative stress.

The studies have been conducted on experimentally induced IR injury in rats with obesity and steatosis. The blood samples were collected before and after induction of reoxygenation; the serum levels of alanine transaminase (ALT), aspartate transaminase (AST) and the hepatic levels of γ-GT, GSH and malondialdehyde (MDA) are estimated.

Treatment with GGsTop decreased the serum ALT and AST levels and markedly inhibited the serum γ-GT activity, it also served in the reduction of hepatic γ-GT, GSH and MDA after ischemia reoxygenation. It also decreased the production of 4-HNE and HMGB1 which are associated with the lipid peroxidation and generation of free radicals and prevented hepatic necrosis during ischemia reoxygenation.

❖ *Lactate Dehydrogenase*

- *The potential utility of Lactate dehydrogenase isoenzymes as a biomarker in determining urinary cytology*

Urine Cytology is a procedure used for screening of urinary tract neoplasms. It had been determined that the combination of urinary cytology and determination of urinary LDH isoenzymes could play an integral role in the diagnosis of bladder tumours. This study aimed to examine the urinary specimens by routine cytology to evaluate the urinary LDH isoenzymes.

Cytological testing of 106 urine samples was conducted to detect the presence of bladder cancer. The amount of LDH-1 in Group 1 was significantly higher than that of Group 2 but the amount of LDH-5 in Group 2 was significantly higher than that of Group 1. The amounts of LDH-3 and LDH-4 in Group 3 were found to be significantly higher than both Groups 1 and 3. Thus a remarkable deviation towards M-fraction was noted in Group 3 in comparison to the other 2 Groups and the M/H ratio in Group 3 was found to be significantly higher than that in Group 1.

The LDH M-fraction of urinary supernatant in positive cytological cases was found to be significantly higher than in

negative cytological cases and in cytologically suspicious cases it was lesser than the positive cases but higher than the cytologically negative cases. There were a few false-negative cases observed in the latter out of which one gave positive results on diagnosing histologically. Additionally, two suspicious cases also turned out to have bladder cancer. It was observed that all three previous cases had a distinctive increase in the isoenzyme M/H ratio.

Thus it was suggested that for diagnosing Urinary tract cancers, determining the LDH isoenzyme is quite useful for both early-stage and post-operative patients having bladder cancer once the inflammation after the surgical resection subsides.

- *A case report on measuring the Serum Level of Lactate Dehydrogenase to study the progression of Multiple Myeloma*

The malignant proliferation of plasma cells in the bone marrow and monoclonal immunoglobulin production are indicators of Multiple Myeloma. The elevation of LDH levels could be an indication of an increase in the level of the tumour, a probable relapse or the occurrence of additional plasmacytomas. To gather more information about the aforementioned claim, a case of an 80-year-old female patient suffering from stage IIIA IgA type multiple myeloma was considered. Melphalan-prednisone (MP) treatment was started to treat multiple myeloma.

Initially, the LDH levels were low during diagnosis and chemotherapy, but as the disease progressed LDH levels rose extraordinarily(7557 U/L) and extramedullary plasmacytomas started to occur which led to the death of the patient. Thus it was concluded that serum LDH levels act as useful biomarkers in the same capacity as that of beta-2 microglobulin and monoclonal immunoglobulin. If the LDH levels are found to be extraordinarily high, after eliminating other causes it can be considered as a case of progression of the disease. High LDH levels are accompanied by the presence of plasmacytomas, thus the entire body examination needs to be conducted at an early stage before the development of any symptoms and so that treatment can be started in advance.

- *The potential diagnostic significance of Lactate Dehydrogenase in Children suffering from Refractory Pneumonia caused by Mycoplasma pneumoniae*

Community-acquired respiratory tract infections in children are often considered to be caused by Mycoplasma Pneumoniae. There is always a risk of the disease developing into a severe or refractory illness, so it is highly desirable to predict the refractory illness promptly so that steroid therapy could be initiated well in advance. With this study, the main aim was to determine whether C-reactive protein, Erythrocyte sedimentation rate(ESR) and Lactate dehydrogenase(LDH) can act as a potential biomarker that can be used to predict refractory M. Pneumoniae pneumonia and determine which candidates may benefit from an early steroid treatment therapy.A real-time, multiplex polymerase chain reaction assay was used to test the nasopharyngeal aspirates for pathogens and a commercial enzyme-linked immunosorbent assay kit was used to detect M. pneumoniae IgM. The refractory M. pneumoniae pneumonia group showed significantly higher levels of serum LDH, C-reactive protein and ESRs in comparison to the normal M. pneumoniae pneumonia group. However, the percentage of lymphocytes in the normal group was found to be higher than in the refractory group. Additionally, it was observed that admission of ESR and serum LDH posed a significant risk factor for refractory M. pneumoniae pneumonia. It was concluded that serum lactate dehydrogenase (LDH) has the potential to act as a biomarker to predict refractory M. Pneumoniae pneumonia and determine which candidates may benefit from an early steroid treatment therapy. It was suggested that further research needs to be conducted to determine the potency of early steroid administration by using LDH as a biomarker to initiate the steroid treatment.

- *Renin*

- *The important role of Renin and Erythropoietin in diagnosing patients suffering from Septic Shock*

Sepsis is the body's extreme response to an infection which involves a slew of biological responses by the nervous, endocrine and immune system. When it comes to hypoxic injuries, Erythropoietin(EPO) is considered to be the response element. A few studies had suggested that Renin may be involved in the upregulation of EPO production. Thus this study aimed to determine the function of EPO and renin as potential biomarkers in case of patients suffering from septic shock.

A total of 44 critically ill patients were evaluated as part of this study. Immunoenzymatic assay was used to determine the concentrations of EPO and Radioimmunoassay was used to determine the concentrations of renin. It was observed that serum EPO levels in nonsurvivors were significantly higher than that of survivors. On admission, even the renin levels were found to be higher in nonsurvivors but the difference between the two did not possess any statistical significance. A negative relation was observed between the blood haemoglobin concentration and serum EPO in the survivors. Additionally, there were significant correlations observed between EPO concentration and SAPS score, PaO2/FiO2 ratio, arterial pH, lactate and renin concentration. For renin, significant correlations were observed only with arterial pH and lactate.

There was an increase in the renin and EPO concentrations in the patients that were admitted due to septic shock. So on the basis of the study, it was concluded that renin may be considered as an important mediator of EPO upregulation in patients suffering from septic shock and further studies regarding the upregulation of EPO expression were required to be conducted.

➢ *The diagnostic significance of Renin in Heart Failure*

Heart Failure is one of the leading causes of death worldwide. An ideal biomarker should be an indicator of the risk that comes with every stage of the disease. Norepinephrine and renin have traditionally been used as biomarkers for HF but to understand the diagnostic and prognostic properties, studies have recommended the use of serum B-type natriuretic peptide (BNP).

A study had reported that norepinephrine and plasma renin activity(PRA) were found to be elevated in patients who died suddenly or of progressive heart failure and in the Vasodilator-Heart Failure Trial II, it was found that they were the prognostic factors. The Valsartan Heart Failure Trial involved a comparative study which showed that both BNP and PRA were efficient prognostic biomarkers of mortality. A study showed that the PRA levels were found to be higher in patients dosed with ACE(Angiotensin-converting enzyme) inhibitors than those not on it. But contrary to that, the patients on β-Blockers were found to have lower PRA levels than the patients not on β-Blockers.

In the case of patients suffering from Heart failure, because of liver congestion, the evidence of PRA is restricted to angiotensinogen levels. So a study suggested that in comparison to PRA levels, direct renin measurement would prove to be a stronger prognostic biomarker in patients with HF. Thus it was concluded that renin still acts an important biomarker for HF and its prognostic ability is still secure especially in real-world situations which involve the use of ARB or ACE inhibitors..

➢ *The Potential Diagnostic significance of Plasma Renin Activity and Plasma Aldosterone Concentration in patients suffering from Sepsis.*

The Renin-Angiotensin-Aldosterone System(RAAS) is essential in preserving the blood pressure homeostasis. Studies in the past have shown that Plasma aldosterone concentrations (PACs) are useful biomarkers in detecting septic shock patients with a high risk of renal dysfunction. But the function of PAC(Plasma aldosterone concentrations) and PRA(Plasma Renin Activity) measurements in the prediction of patients with relation to mortality remains largely unknown which is what this study aimed to evaluate.

105 patients admitted for septic shock were considered for the study for 28 days. Acute Physiology and Chronic Health Evaluation (APACHE) II score and Sequential Organ Failure Assessment (SOFA) score were conducted to determine the severity and prognosis of the disease. Radioimmunoassay was used for PRA and PAC measurements. APACHE II scores and SOFA scores of the survivor group were found to be lower than that of the non-survivor group. Even the PRA and PCAs of the survivor group were found to be significantly lower than that of the non-survivor group.

There was a positive correlation found between the PRA and the SOFA score and the APACHE II score. The PACs were only positively correlated with the SOFA score. Based on multivariate analysis, PRA values ≥3.5 ng ml-1 h-1, coronary arterial occlusive disease, the SOFA score and previous history of cancer all were classified as independent markers of the 28-day mortality. Based on the findings, it was concluded that the elevation of PRA can act as a potential biomarker in stratifying the risk of patients with septic shock and it is an indicator of 28-day mortality.

❖ *Urokinase*

➢ *Evaluation of the Urokinase-Type Plasminogen Activator System on the efficacy of Tamoxifen Therapy in Recurrent Breast Cancer*

Urokinase Plasminogen Activator(uPA) System plays a vital role in facilitating the various steps of Cancer Metastases. Serine protease uPA, its receptor uPAR, and both the plasminogen activator inhibitors- PAI-1 and PAI-2 play a role in prognosticating primary breast cancer. In an earlier study conducted on recurrent breast cancer, it was observed that uPA and PAI-1 levels successfully predicted the efficacy of Tamoxifen therapy. Thus this study served as an extension to the previous study and it involved the evaluation of the uPA system on the efficacy of Tamoxifen therapy.

691 patients with estrogen receptor positive primary breast tumours who had developed a recurrence were considered for the study. The patients were treated with a 40 mg daily dosage of first-line tamoxifen. In the univariate analysis, high tumour levels of uPA and uPAR and PAI-1 were a sign of the failure of the treatment. In the multivariable analysis, additional information over the traditional factors could be obtained from the uPA levels. In the first 9 months of follow-up, when the relationship of the 4 factors with Progression-Free Survival was analysed, high levels of uPA, uPAR and PAI-1 indicated a shorter PFS. But during the analysis without any time restriction, it was observed that high PAI-2 level was a sign of a longer benefit from Tamoxifen therapy.

In the study, it was observed that in the case of tamoxifen treatment of recurrent breast cancer, uPA and PAI-2 levels were found to be strong predictive factors for clinical benefit and length of response respectively. It was concluded that the tumour levels of the four factors of the uPA system might play a valuable role in designing future individualised uPA system targeted therapy methods and that further studies were needed to support and confirm the findings.

➢ *Potential Therapeutic Strategy to treat Aggressive Cancers by hindering the interactions between Urokinase receptor(uPAR) and its co-receptors*

The spread of cancer cells from the primary site to any other location follows a complex sequence which includes the detachment of the tumour cells from primary tumours,

degradation of extracellular matrix (ECM), intravasation into the bloodstream, extravasation from the circulation and finally settling in a distant organ. The various steps of Cancer metastasis are facilitated by Urokinase receptor (uPAR). In various types of cancer, uPAR is often found to be elevated and it normally signifies a poor patient prognosis.

Endocytosis and recycling of uPAR are observed to have a complex role in the function of uPAR as these are the crucial events that regulate the distribution and level of uPAR along the plasma membrane. Since uPAR is an anchored cell surface protein, the relaying of the downstream signals was found to be carried out by co-receptors. These interactions with the co-receptors play a crucial role to facilitate the development and progression of the tumour by eliciting cell migration, invasion, ECM proteolysis and EMT. In mostly all types of aggressive cancers, uPAR is found to be overexpressed and it plays a crucial role in tumour progression and metastasis. Earlier studies focused mainly on blocking the uPA-uPAR binding. Apart from the proteolysis functions of uPAR, uPAR also functions independently from proteolysis. Since the co-receptors play an important role in the non-proteolytic activity of uPAR, hindering the interactions between them was suggested as a potential strategy to target these aggressive malignancies.

➢ *A novel prognostic biomarker to detect the presence of Cardiovascular Diseases*

Urokinase-type plasminogen activator receptor (uPAR), a type of protein found in several types of cell, had not only been correlated to traditional biomarkers but was also able to outperform CRP in predicting Cardiovascular disease(CVD). Though the physiological functions of soluble forms of urokinase-type plasminogen activator receptor(suPAR) were still not completely understood, it was observed that there was a correlation between suPAR levels and tumour necrosis factor.

The investigation for the predictive value of suPAR in CVD was conducted on the Danish MONICA 10 cohort and it was observed that there was an elevation in the suPAR levels which was an indicator of Type 2 diabetes mellitus, increased risk of cancer, CVD and death. In the case of patients already suffering from CVD, it was observed that there was an elevation in the plasma suPAR levels, which could be used to detect the presence and the severity of Coronary Artery Disease. Additionally, in cases of cerebrovascular diseases, the suPAR levels were found to predict the increased incidence of carotid plaque and ischemic stroke.

Based on the available literature, it was concluded that sUPAR is a promising prognostic biomarker that helps in identifying organ dysfunction and chronic inflammation. But it remains a mystery whether suPAR has a causal role in CVD or is just merely a biomarker. Additionally, the regulation mechanism of suPAR and its interaction with other biomarkers needed to be understood better to gain an understanding of its lack of association with obesity and the higher levels obtained in women than in men. Lastly, no cut-off values had been established and there were no existing therapies that specifically targeted the suPAR levels. Hence further research and studies have to be conducted before it is employed in clinical practice.

❖ *Acid Phosphatase*

➢ *Fundamentals of Acid phosphatase*

Acid phosphatase is an enzyme which catalyzes the hydrolysis of certain phosphate esters in an acidic setting. As these play a major role across several biological kingdoms, in humans Acid Phosphatase (AP) is used to clear out various disparities in the test to know about diverse physiological conditions in the human body AP is a vague marker as it is secreted from various tissue in the human body. If found in higher levels; AP can be used as selective markers in some cases like osteoporosis and Prostate cancer. In Prostate Cancer, the cancerous prostate cells produce more amounts of AP than normal prostate cells, which makes it a significant serum marker for a long time later replaced by prostate-specific antigen (PSA). Prostate AP is also seen in Seminal fluid which makes it easy to determine semen in forensic tests. In the case of osteoporosis, the bones produce TRAP(Tartrate Resistant AP) which is a good serum marker for diagnostics than other methods but it is still in development. TRAP can also be used to diagnose Hairy cell leukaemia (HCL). AP had gained greater interest as a therapeutic target for immunotherapy. AP can be used as a targeted vaccine to specific cancerous areas to reduce the expression of cancerous cells in Prostate cancer. A developing DNA vaccine for prostate cancer has shown promising results known as Provenge vaccine. There is a large scope for the Provenge/ sipuleucel-t vaccine treatments in future as it requires more team and financial effort.

➢ *The necessity of Acid phosphatase 2 (ACP 2) in the course of viral influenza entry*

The influenza viruses have been a rattling problem of human history for the past few decades. The virus belongs to the Orthomyxoviridae family. This study is to identify the role of Acid Phosphatase 2 (ACP2). The lysosomal acid protein ACP2 is traced in both the lysosome and the extracellular environment as most steps for the entry of influenza virus takes place in endolysosomes, but ACP2 is not that vital for the fastening step of influenza virus entry. On the examination of ACP2 specific siRNA by western blot and quantitative reverse transcription on the affected cells, the researchers found out that ACP2 is essential for Influenza A virus(IAV). Investigator had used an assay depending on fluorescence which relies on certain lipophilic dyes to find out about the role of ACP2 in the membrane fusion step of influenza entry. In the following tests and previous conclusions, the reduction of ACP2 repressed fusion while entering the Influenza B virus which concludes the essentiality of ACP2 for the fusion of membranes at the time of the entry of IAV and IBV. Then the

researchers had chosen to experiment the findings across various spectrum of viruses as such they had selected Ebola and Hepatitis c virus because of undergoing similar membrane fusion depending on pH. By doing various tests on Hepatitis C and Ebola, the researchers arrived at an outcome that ACP2 is not needed for the replication of other viruses than the IAV and IBV. By these findings, the analysts concluded that by silencing the gene focused on ACP2 in Human genome for the inhibition of viral replication of Influenza viruses and to use ACP2 as a lysosomal marker in various tests to find out about influenza.

➢ *Human Prostatic Acid Phosphatase: -- The novel roles*

Prostate cancer is a major prevailing non-skin cancer found in men. It has been a long time since the PAP have been used for diagnosis as the increased level of PAP is seen in cancer-specific prostate cells. PAP predicts the cancer-specific survival of patients and PAP had known to reduce chronic pain in patients. Also, it is a breakthrough with the development of Sipuleucel T vaccine(Provenge vaccine) for the diagnosis of prostate cancer. PSA test had been a preferred marker to diagnose several patients of prostate cancer since the start of the 21st century.

Human PAP is a secreted glycoprotein enzyme which is synthesized in the epithelial cells of prostate glands. Provenge vaccine based on the regulation of PAP gene expression which identifies and kills the more PAP producing cancer cells thus increasing the chances of survival and cure. PAP has a prostate physiological role as the PAP is seen profusely in the Seminal fluid. PAP in the semen help in increased mobility of sperms which correlates being a major part of fertilization. In previous studies, it is concluded that the concentration of PAP in semen is inversely associated with sperms thus PAP is a mood marker for diagnosing oligospermia. PAP play a major role in the transmission of the deadly virus HIV, Thus the researchers found out that PAP promotes the enhancement of HIV and other viruses like XMRV(Semen-derived enhancer of viral infection (SEVI)), which gives a conclusion to use compact methods to inhibit the fibrillization of SEVI to regulate the expression of viruses like HIV.

❖ *Alkaline Phosphatase*

➢ *The fundamentals of Alkaline phosphatase:-*

Alkaline phosphatase (ALP) corresponds to a group of enzymes which help in the removal of the phosphate group from molecules like nucleotides and protein. ALP is found in higher concentration in liver and bone; lower concentrations in kidney tubules, intestinal epithelium, lung & placenta. Despite ALP being present in many tissues all around the body, their actual physiological function remains largely unknown. ALP serum levels vary with age in healthy individuals. Levels are high in the body while in childhood and puberty due to bone growth and development. Then the next noticeable rise in serum levels is seen in old age. ALP is an enzyme which has a half-life of 7 days and the clearance from serum is autonomous of bile duct patency or the liver's functional capacity. The increased level of alkaline phosphatase in hepatobiliary disorders has been a matter of discussion. Studies report that vesicles having alkaline phosphatase and more such are bound to sinusoidal membranes which are found in patients with cholestasis as the ALP starts its production in response to biliary obstructions, the serum levels may be normal in the early phase of acute biliary obstruction despite the fact that serum aminotransferases are at its peak. In the tests to diagnose ALP level the most preferred one is to check its ability of the enzyme to hydrolyze other phosphate esters in principle. When ALP becomes elevated in the liver biochemical test as compared to other constituents, the practitioner should identify the underlying cause of conditions and also the source of extra secretion of ALP. ALP based test can be used to diagnose cholestasis, biliary obstruction caused by the cancers, choledocholithiasis, sclerosing cholangitis drug-induced liver injury, chronic rejection of liver, allografts and infiltrative liver disease. Some activities of some pathogens can also be traced from ALP tests (AIDS, Tuberculosis .etc.) The higher levels of ALP gives a hint to chronic conditions like chronic hepatitis, viral hepatitis, congenital heart failure and liver cirrhosis. Therefore it is necessary to include ALP test in primary healthcare as it gives a clue about underlying conditions closely or remotely related to the liver.

➢ *The important roles of Intestinal alkaline phosphatase*

Alkaline phosphatases belong to the suborder of enzymes with numerous activities including hydrolases, isomerases & transferases which catalyzes the monoesters of phosphoric acid & transphosphorylation. The activity of intestinal ALP helps in lipid absorption in the intestine across the enterocyte apical membrane. IAP is capable of hydrolyzing different substrates provided by the diet and polyphosphates and most phosphate residues of nucleotides. IAP is removed by glycoprotein in the apical membrane by a glycosylphosphatidylinositol linkage. IAP controls the lipid absorption in intestines as the fatty acids and glycerol are absorbed by the enterocyte which is being hydrolyzed from triglycerides. Fat absorption by enterocytes induces a faster and accurate internalization of the apical membrane-anchored IAP. IAP is also associated with the regulation of duodenal bicarbonate secretion and surface pH which comes from the fact that IAP is readily found in duodenum having a pH optimal (>8) range for the same. IAP helps in the dephosphorylation of bacterial LPS (lipopolysaccharides) which reduce its toxicity as the LPS is a major product of gram-negative bacteria found in intestines.On a recent test, two independent mice were selected (one based on systemic and the other mice based on intestinal) to demonstrate the effects of IAP in the transmucosal passage of bacteria. This allowed the researchers to demonstrate that IAP deficient mice experienced greater bacterial passage from the intestinal lumen to mesenteric lymph nodes than the wild type which gives an outlook that IAP decreases the transmucosal passage of bacteria and protects the intestine from LPS induced

ISSN No:-2456-2165

inflammation. IAP creates an intestinal tolerance to commensal bacteria. IAP also keeps control of inflammations and sepsis especially related to LPS caused sepsis. This gives greater hope in future treatments if this regulation can be promoted to usefulness in healthcare by effective therapeutic methods. Traces of IAP based activity can be seen in diet patterns as its implication are seen as important in terms of maintaining nutrition(obese) and health(sepsis) for the elderly population. Also, some sources are citing that macronutrients modulate the IAP to some extent. All these conclude to have IAP based tests and treatments especially for emergency care (sepsis) and intestinal issues.

➢ *Response of Alkaline phosphatase in polycystic liver disease while somatostatin analogue therapy*

Polycystic liver disease(PLD) is mostly seen in patients with autosomal dominant polycystic liver disease and autosomal dominant polycystic kidney disease. The condition caused by PLD causes chronic symptoms and also reduces the quality of life. Here researchers conducted some analysis of patients in the Mayo clinic to reach their conclusions. Their primary outcome was dependent on the change in liver volume at the end of therapy compared to the baseline were calculated on different time points. The secondary outcome was based on the change in kidney volume and change severity of symptoms measured in the same therapy as an independent variable. The conclusion the researchers had arrived are i)the elevated alkaline phosphatase level predicts the volume response of the liver in polycystic liver disease during somatostatin analogue therapy ii)in the somatostatin analogue therapy type, underlying diagnosis and baseline renal function does not affect the volume response of the liver. iii) The reduction in gastrointestinal symptoms is seen with somatostatin analogue therapy. The conclusion researchers arrived at is alkaline phosphatase could serve as a liver-specific biomarker in response to the polycystic liver disease for patients awaiting somatostatin analogue therapy.

❖ *Alanine Aminotransferase*

➢ *Fundamentals of Alanine Aminotransferase*

The liver is considered at the topmost in the list of vital organs of a human body as its secretion bile produces plasma proteins which regulate the composition of blood plasma, this helps in the transfer of energy and nutrients, plasma detoxification and excretory process of the body. For proper diagnostics of medical history hepatic enzymes are tested in which the test mainly circulates around Alanine Aminotransferase (ALT) & Aspartate Aminotransferase(AST). The elevation in transaminases gives an outlook about the diagnosis-related. All patients with higher transaminases are not necessarily a hepatic patient but are prone to hepatic conditions. Researchers had also found out that the serum levels may vary according to gender and ethnicity. Pathological & lifestyle causes shows a noticeable increment in serum levels of ALT & AST. Thus ALT & AST levels can be used as a marker for diagnosis of Non alcoholic fatty liver diseases, steatohepatitis, chronic hepatitis, autoimmune hepatitis, Alpha-1 antitrypsin deficiency, drug-induced liver injury, acute viral hepatitis and genetic disorders affecting the liver. The transfer of amino groups from the alanine to alpha-ketoglutarate is catalyzed by ALT enzyme giving out the conversion products L glutamate & pyruvate. This is a vital process for the tricarboxylic acid cycle(TCA). ALT is found all across the human body but the highest tissue concentration of ALT Activity is found in the cytosol of hepatocytes. The half-life of ALT is roughly around 47 hours. Hepatic function panels access ALT through blood samples. Liver function test is widely used among various labs to test ALT and AST levels. If the Alt level is higher than 1000U/L the patient may be diagnosed for acute ischemic liver injury, severe drug-induced liver injury, acute viral hepatitis. NAFLD can also be determined by the test if the GGT levels are also increased in the serum. Overall the levels of ALT in the body determine various conditions of the liver.

➢ *The connection among alanine transferase and growth hormone*

Growth hormone is the key regulator of fat metabolism which also targets the liver. Clinical research had found out that the liver GH receptor is a cause for NAFLD as the liver GH receptor could increase intracellular lipid accumulation. Also, there are some studies on the relationship between liver enzymes and GH in short children and adolescents caused by GH deficiency (GHD). As ALT being one of the major enzymes produced by the liver, it is of relevance here to check its association with growth hormone. More fair approach to check the body's GH status is to check the GH peak after the stimulation.

The research takes place in 670 children with the short structure to have a more clear understanding of the aim. The research concludes in a positive manner as it showed GH peak is independently related to ALT levels after adding some legible parameters. GH secretion level was strongly interconnected with ALT in short children and adolescents which gives a suggestion for them to screen for NAFLD and use rhGH based treatment for short structure.

➢ *Alanine Aminotransferase - The helpful pointer of health and disease.*

Physicians across the world have been aware of liver enzyme measurements of ALT and AST in the diagnosis of liver disease; but it is also a good indicator to determine obesity, metabolic syndrome, presence of cardiovascular disease and NAFLD. ALT is an enzyme that catalyzes the process of transfer of amino groups to form haptic metabolite oxaloacetate. ALT has a half-life of about 47 hours while AST has a half-life of 17hours. A small injury in the liver could rise the ALT levels to a peak which makes it a good marker for liver diseases. ALT test may be also used as a substitute for a blood test in some cases as it provides BMI and triglyceride levels. Increased levels of ALT may also contrast the severity of NAFLD and ALD. The frequent checkup on ALT

elevations and routine blood tests may give a clue for Hepatitis C Virus diagnosis even though ALT levels alternate in the infection period. Hepatitis B Virus causes a chronic liver infection which is often asymptomatic but often found out by testing the ALT levels; Also these tests can determine the course of the natural history of the infection. ALT is also a marker for diagnosing drug-induced hepatotoxicity, autoimmune liver diseases, cholestatic liver diseases and metabolic liver diseases. ALT may also serve as a marker for measuring the overall health and mortality risks as ALT could predict underlying liver, cardiovascular and metabolic conditions. Testing of ALT has been a good selective test by physicians for a long time therefore it should be incorporated in primary routine checkup of patients.

- *The foundations of LFT (Liver function test)*

Liver is a vital organ which has an important role in metabolism & regulation of blood cells. LFT is a good test to evaluate the condition of the liver. It checks the levels of alanine transaminase (ALT) and aspartate transaminase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), serum bilirubin, prothrombin time (PT), the international normalized ratio (INR) and albumin in the liver When the levels of ALT and AST boosts out of proportion to ALP and bilirubin signifies a hepatocellular disease. Whereas, When the levels of ALP and bilirubin are disproportionate to ALT and AST would signify a cholestatic pattern. For proper diagnosis of Hepatocellular pattern, the labs would check whether ALT or AST is predominant in outcome by which the physician determines the disease. Acute or chronic viral hepatitis, steatohepatitis, acute Budd-Chiari syndrome, ischemic hepatitis, autoimmune, hemochromatosis, medications/toxins, autoimmune, alpha1- antitrypsin deficiency, Wilson disease, Celiac disease are the clues for diagnosis for ALT predominant tests. Alcohol-related, steatohepatitis, cirrhosis, non-hepatic are the clues for the diagnosis for AST predominant tests. In the diagnosis of cholestatic pattern, levels of alkaline phosphatase, GGT, bilirubin are checked by the labs from which the cause is determined by the physician. Bile duct obstruction, primary biliary cirrhosis, primary sclerosing cholangitis, medication-induced, infiltrating diseases of the liver (sarcoidosis, amyloidosis, lymphoma, among others), cystic fibrosis, hepatic metastasis, cholestasis are clues for Hepatobiliary causes. Bile duct obstruction, primary biliary cirrhosis, primary sclerosing cholangitis, medication-induced, infiltrating diseases of the liver (sarcoidosis, amyloidosis, lymphoma, among others), cystic fibrosis, hepatic metastasis, cholestasis are the clues when the non-hepatic cause of the rise in the alkaline phosphatase levels. ALT and AST(aminotransferases) levels are tested as they are good selective markers for hepatocellular injury.

ALT and AST are normally found in a bit higher concentrations in males than females. Alkaline phosphatases are normally found in higher concentrations near microvilli. ALP is found in higher concentration in the growth phase of children due to the increased osteoblastic activity. Also, the normal reference age for females is a bit higher than that of males. Glycoprotein gamma-glutamyl transferase (GGT) is seen on membranes of cells with high secretory or absorptive activities as its levels are more specific to biliary diseases. GGT is seen normally in high levels in infants. The final catabolism product of heme, Bilirubin is also analyzed in LFT. Albumin is produced by liver 10gms per day, reduction or overproduction in the rate gives a clue about the liver disease but if liver functions normally while albumin levels are low it is a sign of malnutrition. Prothrombin time is the rate of conversion prothrombin to thrombin. When AST to ALT ratio is 2:1 it predicts the case of alcoholism. Most medications are prone to causing Acute hepatocellular injury, chronic hepatocellular and/or cholestatic liver damage. In the case of viral hepatitis, elevation in LFTs is seen. Autoimmune hepatitis also shows prolonged ups and down in LFTs due to the autoimmune damage caused to the liver. Hemochromatosis, Wilson Disease and Alpha-1 Antitrypsin Deficiency can also be analyzed with LFTs.

II. CONCLUSION

The review shows the usage of these enzymes as diagnostic reagents and potential clinical and therapeutic applications

ACKNOWLEDGEMENT

We would like to thank our supervisor, Professor Bharat Kwatra, from Invenzion Labs. Whose expertise was invaluable in formulating the research questions, methodology and drawing Conclusions. His insightful feedback and Guidance pushed us to sharpen our thinking and brought our work to a higher level.

REFERENCES

[1]. Kwatra, B. A review on Potential properties and therapeutic Applications of Carrots and Their Seed Extracts.
[2]. Kwatra, B. & Mudgil, M. Finding Closely Related Organisms to Homo sapiens Insulin; Using Computational Biology.
[3]. Aldrich, F. D. Cholinesterase assays: Their usefulness in diagnosis of anticholinesterase intoxications. *Clin. Toxicol.* **2**, (1969).
[4]. Nishikawa, A., Tanaka, T., Takeuchi, T., Fujihiro, S. & Mori, H. The diagnostic significance of lactate dehydrogenase isoenzymes in urinary cytology. *Br. J. Cancer* **63**, (1991).
[5]. Mustazza, C. *et al.* Synthesis and cholinesterase activity of phenylcarbamates related to Rivastigmine, a therapeutic agent for Alzheimer's disease. *Eur. J. Med. Chem.* **37**, (2002).
[6]. Richter, J. G. *et al.* Differential diagnosis of high serum creatine kinase levels in systemic lupus erythematosus.

Rheumatol. Int. **23**, (2003).
[7]. Meijer-Van Gelder, M. E. *et al.* Urokinase-type plasminogen activator system in breast cancer: Association with tamoxifen therapy in recurrent disease. *Cancer Res.* **64**, (2004).
[8]. Tamion, F. *et al.* Erythropoietin and renin as biological markers in critically ill patients. *Crit. Care* **8**, (2004).
[9]. Cummings, J. L. Use of Cholinesterase Inhibitors in Clinical Practice: Evidence-Based Recommendations. *Focus (Madison).* **2**, (2004).
[10]. Franceschino, A., Tornaghi, L., Benemacher, V., Assouline, S. & Gambacorti-Passerini, C. Alterations in creatine kinase, phosphate and lipid values in patients with chronic myeloid leukemia during treatment with imatinib. *Haematologica* **93**, (2008).
[11]. Kim, W. R., Flamm, S. L., Di Bisceglie, A. M. & Bodenheimer, H. C. Serum activity of alanine aminotransferase (ALT) as an indicator of health and disease. *Hepatology* vol. 47 (2008).
[12]. Lallès, J. P. Intestinal alkaline phosphatase: Multiple biological roles in maintenance of intestinal homeostasis and modulation by diet. *Nutrition Reviews* vol. 68 (2010).
[13]. Noh, H., Hong, S. & Huang, S. Role of urokinase receptor in tumor progression and development. *Theranostics* vol. 3 (2013).
[14]. Kong, H. Y. & Byun, J. Emerging roles of human prostatic acid phosphatase. *Biomolecules and Therapeutics* vol. 21 (2013).
[15]. Teke, H. Ü., Başak, M., Teke, D. & Kanbay, M. Serum level of lactate dehydrogenase is a useful clinical marker to monitor progressive multiple myeloma diseases: A case report. *Turkish J. Hematol.* **31**, (2014).
[16]. Sato, Y. Renin – A historical biomarker of heart failure – . *Circulation Journal* vol. 79 (2015).
[17]. Lu, A., Wang, C., Zhang, X., Wang, L. & Qian, L. Lactate dehydrogenase as a biomarker for prediction of refractory mycoplasma pneumoniae pneumonia in children. *Respir. Care* **60**, (2015).
[18]. Cyrille, N., Villablanca, P. & Ramakrishna, H. Soluble urokinase plasminogen activation receptor-An emerging new biomarker of cardiovascular disease and critical illness. *Ann. Card. Anaesth.* **19**, (2016).
[19]. Gevers, T. J. G., Nevens, F., Torres, V. E., Hogan, M. C. & Drenth, J. P. H. Alkaline phosphatase predicts response in polycystic liver disease during somatostatin analogue therapy: A pooled analysis. *Liver Int.* **36**, (2016).
[20]. Kwatra, B. Tinospora Crispa As A Future Cure For Obesity/Cholesterol. *Int. J. Sci. Technol. Res.* **6**, 340–341 (2017).
[21]. Kwatra, B. HACKING THE BLOOD-BRAIN BARRIER. *Eur. J. Biol. Med. Sci. Res.* **5**, 10–13 (2017).
[22]. Kwatra, B. LOCATOR THEORY FOR ELE-MENTS IN PERIODIC TABLE 'LEPT'. *Glob. J. Pure Appl. Chem. Res.* **5**, 9–10 (2017).
[23]. Chung, K. S. *et al.* Implications of Plasma Renin Activity and Plasma Aldosterone Concentration in Critically Ill Patients with Septic Shock. *Korean J. Crit. Care Med.* **32**, (2017).
[24]. Lee, J., Kim, J., Son, K., D'Alexandry D'Orengiani, A. L. P. H. & Min, J. Y. Acid phosphatase 2 (ACP2) is required for membrane fusion during influenza virus entry. *Sci. Rep.* **7**, (2017).
[25]. Li, Q. *et al.* Donepezil-based multi-functional cholinesterase inhibitors for treatment of Alzheimer's disease. *European Journal of Medicinal Chemistry* vol. 158 (2018).
[26]. Mudgil, M. & Kwatra, B. Mosquito Menace Aim: Observing the life cycle of Aedes aegypti mosquito and understanding its behavior towards different natural oils for encouraging natural methods of repellence. *Int J Sci Res [Internet]* **8**, 1314–1315 (2019).
[27]. Kwatra, B. Studies on People Employed in High Risk Workplace: Between Genetic Polymorphism for Tumor Necrosis Factor (TNF-A) and Blood Pressure. *J. Pharm. Pharm. Sci* **8**, 488–500 (2019).
[28]. Bharat Kwatra, M. M. Mosquito Menace. *Int. J. Sci. Res.* **8**, 1314–1315 (2019).
[29]. Kwatra, B. MECHANISMS OF PATTERN FORMATION OF FBP17 IN MAST CELLS. *Int. J. Adv. Res.* **7**, 413–414 (2019).
[30]. Kwatra, B. Allicin -An After Digestion Antimicrobial Agent. *ACTA Sci. Microbiol.* **2**, 48–51 (2019).
[31]. Kwatra, B. Procuring Natural Dye for Solar Cell Using Leaf Waste. *Int. J. Sci. Res.* **7**, 46–47 (2019).
[32]. Kwatra, B. Holothuroidea (Sea Cucumber): Key to Anti-Aging. *Int. J. Sci. Res.* **8**, 884 (2019).
[33]. Kwatra, B. Bioactive-Compounds: an alternative to control Candida spp. *Int. J. Sci. Res. Rev.* **8**, 221–223 (2019).
[34]. Bharat Kwatra, C. A. Mathematical and Statistical Approach to Define Past Present Future Events. *International Journal of Science and Research (IJSR)* vol. 8 260–263 (2019).
[35]. Kwatra, B. Effects of Mineral Separation by Time and Enteric Coating Mechanism for Calcium and Iron Absorption in Mammalia. *Int. J. Sci. Res.* **8**, 1265–1270 (2019).
[36]. Kwatra, B. CALCIUM AND IRON ABSORPTION: INVITRO STUDIES. *Int. J. Med. Biomed. Stud.* **3**, 59–61 (2019).
[37]. Kwatra, B. A REVIEW ON POTENTIAL PROPERTIES AND THERAPEUTIC APPLICATIONS OF BROMELAIN. *WORLD J. Pharm. Pharm. Sci.* **8**, 488–500 (2019).
[38]. Kwatra, B. EXPRESSION AND CHARACTERIZATION IN PICHIA PASTORIS BY CLONING OF DELTA 4 DESATURASE FROM ISOCHRYSIS GALBANA. *Indian J. Appl. Res.* **9**, 1–2 (2019).

[39]. Kwatra, B. & Mudgil, M. PROTONATED CRAB SHELL WASTE AS FUNGAL INHIBITOR. *Int. J. Med. Biomed. Stud.* **3**, 111–116 (2019).
[40]. Kwatra, B. HYDROQUINONE: A novel growth inhibitor and apoptosis inducer in U-251 MG CELLS. *Int. J. Med. Biomed. Stud.* **3**, 15–16 (2019).
[41]. Ji, B. *et al.* Association between Alanine Aminotransferase and Growth Hormone: A Retrospective Cohort Study of Short Children and Adolescents. *Biomed Res. Int.* **2019**, (2019).
[42]. Kwatra, B., Hussain, M. S., Bhowmik, R. & Manoharan, S. Reviewing Therapeutic and Immuno-Pathological Applications of Vitamins and Carotenoids. *Int. J. Sci. Res. Sci. Technol.* **7**, 287–313 (2020).
[43]. Kwatra, B. & Modi, R. Therapeutic Potentials and Applications of Folic Acid and Beta Carotene. *Int. J. Sci. Res. Sci. Technol.* **7**, 271–282 (2020).
[44]. Kwatra, B. UTERINE CANCER: SEX DOMINANT CHARACTER. *Int. J. Adv. Res.* **8**, 663–667 (2020).
[45]. Kwatra, B. Candidate genes of OCD interact with human retrovirus to form new links in inflammatory hypotheses. *Int. J. Sci. Appl. Res.* **7**, 1–2 (2020).
[46]. Kwatra, B. & Arora, C. Investigation of Conductance Quantization with Magnetic Field Control and Application of Biosensor. *Int. J. Innov. Res. Technol.* **6**, 271–272 (2020).
[47]. Kwatra, B. COLLAGEN SUPPLEMENTATION: THERAPY FOR THE PREVENTION AND TREATMENT OF OSTEOPOROSIS AND OSTEOARTHRITIS: A REVIEW. *WORLD J. Pharm. Pharm. Sci.* **10**, 589–604 (2020).
[48]. Bharat Kwatra, C. A. Studying the movements of the resonant elastic pendulum. *Indian J. Appl. Res.* **10**, 1–2 (2020).
[49]. Bharat Kwatra, C. A. THE UNEXPLAINED SIMILARITY BETWEEN THE ATOMIC AND GRAVITATIONAL MODELS. *Int. J. Adv. Res.* **8**, 1099–1107 (2020).
[50]. Kwatra, B. & Mudgil, M. LIGHT ASSISTED TIO2-BASED NANOCOMPOSITE SYSTEMS: A NOVEL TREATMENT FOR CANCER. *Int. J. Med. Biomed. Stud.* **4**, 28–32 (2020).
[51]. Kwatra, B. A Review on Potential Properties and Therapeutic Applications of Vitamin D. *Int. J. Sci. Res.* **9**, 682–691 (2020).
[52]. Kwatra, B. Maprovit 3, 6, 9: Perfect Companion of your Immune System to Fight Corona Virus Hit. *Int. J. Sci. Res.* **9**, 241 (2020).
[53]. Bharat Kwatra, M. M. Untangling the Mathematical Relation Between Natural Selection and Adaptive Radiation Using Macromolecules and Microevolutionary Forces. *Int. J. Res. Sci. Technol.* **7**, 313–339 (2020).
[54]. Ali, A. H. *et al.* Open-label prospective therapeutic clinical trials: oral vancomycin in children and adults with primary sclerosing cholangitis. *Scand. J. Gastroenterol.* (2020) doi:10.1080/00365521.2020.1787501.
[55]. Villén, N. *et al.* Multimorbidity patterns, polypharmacy and their association with liver and kidney abnormalities in people over 65 years of age: A longitudinal study. *BMC Geriatr.* **20**, (2020).
[56]. Kubota, R., Hayashi, N., Tsuchishima, M., Tsutsumi, M. & George, J. Inhibition of gamma-glutamyl transpeptidase ameliorates hepatic/reperfusion injury in rats with fatty liver. *J. Hepatol.* **73**, (2020).
[57]. Doyno, C., Sobieraj, D. M. & Baker, W. L. Toxicity of chloroquine and hydroxychloroquine following therapeutic use or overdose. *Clinical Toxicology* (2020) doi:10.1080/15563650.2020.1817479.