Biomarker Application in Forensic Biology and Clinical Research: Enhancing Sensitivity and Specificity in Risk Assessment.

G V Sai Soumya[1], Harishkumar T. Khubchandani[2], Ankita Patel[1], Kapil Kumar[1], Rushikesh Joshi[1]*

[1]*Department of Biochemistry & Forensic Science, University School of Sciences, Gujarat University, Ahmedabad, Gujarat, India*

[2]*Department of Forensic Medicine and Toxicology, B. J. Medical College, Ahmedabad, Gujarat, India.*

***Corresponding Author**

Dr. Rushikesh Joshi,

Department of Biochemistry and Forensic Science,

University School of Sciences, Gujarat University, Ahmedabad 380009, Gujarat, India.

Abstract:

Biomarkers are the indicators that are measured by normal organic process, pathogenic process, and many other processes. The knowledge of biology when applied to solve a crime is called forensic biology. The study of biological evidence and living things collected at crime scenes is known as forensic biology. Biomarkers are also used in basic research and clinical research. The use of biomarkers enhances the sensitivity and specificity of the exposure to risks. Each organ in the human body tends to release a specific hormone which in biological terms is called a biomarker. The organ which is considered is the brain, heart, kidney, and liver. Not only organs but also there are body fluids which also have the presence of some markers which are sweat, saliva, synovial fluid, amniotic fluid, seminal fluid, and vitreous humour. There are around 250 biomarkers in the human body which are measured by various assay methodologies. The combined function of all these biomarkers helps in maintaining the stability and build-up of the metabolism in the human body and any changes in them indicate an improper function in the human system.

Keywords: *biomarkers, forensics, biology, organs, body fluids.*

I. INTRODUCTION

A biomarker is a feature that has been specified and is assessed as a measure of a pathogenic process, a normal organic process, or a reaction to exposure or an intervention, including a therapeutic intervention. Working with biomarkers in epidemiological studies increases the validity by lowering measurement bias for neurological diseases. The sensitivity and specificity of risk factor exposures are enhanced by the use of biomarkers. Molecular biomarkers offer the added ability to identify those who are disease prone. Neurological practicing has already been impacted by molecular genetics, leading to more accurate diagnosis [1]. By definition, a biomarker is a characteristic that may be scientifically analysed and evaluated as a sign of beneficial biological activities, detrimental biological processes, or pharmacological responses to therapeutic interventions. An ideal biomarker possesses particular qualities that make it suitable for evaluating a particular illness condition [2]. A vast subcategory of medical signals is referred to as biomarkers, a portmanteau of the word's biological markers. Biomarkers are employed in both scientific and applied research. In addition to being utilised for pharmacodynamic and dose-response investigations, they may be employed for illness screening, diagnosis, characterisation, and monitoring as prognostic markers for generating individualised treatment interventions. Today, the use of biomarkers in fundamental, clinical, and clinical trial research is virtually universally acknowledged. The validity of biomarkers is frequently taken for granted when it should really be continuously and repeatedly assessed. The word "biomarker" refers to a large subcategory of medical symptoms that are an objective indicator of the medical status viewed from outside the patient and that may be quantified reliably and reproducibly.

The field of forensic science includes forensic biology as a subfield. It uses biological expertise to recognize and examine biological evidence gathered from the crime scene, the victim, or the suspect to prove that the crime occurred. The study of evidence relating to living things and the biological materials they are typically discovered with at crime scenes is the focus of the subject of forensic biology. The post-mortem interval, also known as the time of death, is a crucial stage in the majority of homicide investigations as well as unexpected fatalities (including hospital deaths), and it continues to be one of the variables that is hardest to define and establish, despite the extensive searches that have been made. After death, the body experiences a variety of physical and chemical changes that progress gradually until the body is fully decomposed. These physicochemical changes are typically described in terms of the five phases of decomposition: advanced decay, eroded, fast decay, and dehydrated residue. The timing of these changes is the point at which PMI

identification becomes difficult since the character or existence of each decomposition stage is significantly impacted by endogenous and environmental variables such temperature, humidity, age of the deceased, drug use, and disease. However, different stages of decomposition occur in all decaying bodies. [3][4]. Recent developments in biochemical methods, which are more sensitive and precise than conventional observation methods such as the detection of rigour, algor mortis, and putrefaction phases, have made high throughput operations conceivable [3] [5].

This review has a focus on the biochemical markers which are released from various organs in a human body and various techniques and methodologies for estimation of the time since death by using various markers.

Sr. no.	**Organs and body fluids**	**Biomarkers**
1.	Kidney	Creatinine, urea cysteine C and β trace protein
2.	Heart	Cardiac troponin, creatine kinase, CK-MB, and myoglobin
3.	Liver	Bile, albumin
4.	Brain (Cerebro spinal fluid)	Electrolytes, Carbohydrates, Non-protein nitrogen compound, Proteins.
5.	Sweat	Determination of sweat chloride concentration.
6.	Saliva	Salivary proteomics
7.	Synovial fluid	Commonly performed biochemistry test is glucose level.
8.	Amniotic fluid	Amniotic fluids bilirubin is an indirect method.
9.	Seminal fluid	Spermatozoa, fructose, alpha-glycosidase and glycerylphoyl choline, acid phosphatase.
10.	Vitreous humour	Fructose amine, ketone bodies, lactate, and glucose levels.

Table 1:Organs and body fluids which release specific biomarkers.

Biomarkers are important in both drug development process and the larger biomedical research enterprise[6]. Estimating the post-mortem interval (PMI) is one of the most crucial and difficult problems in forensic pathology. The time since death (TSD), also known as PMI, is the amount of time that has passed since a person has passed away.

A complex set of pathogenic and physiological processes significantly affect the structure and make-up of the physical body after death. These approaches are mostly supported by physical physiochemical progressive changes, such as body chilling, livor mortis, and

rigour mortis, in early PMI the commencement of microbial proliferations. In forensic pathology, post-mortem chemistry, also known as thanato chemistry, has been established to help with more accurate TSD estimates and to provide useful information regarding the cause of death. According to Coe[7] "one of the more important axillary procedures for the forensic pathologist" is forensic chemistry. Studies on post-mortem chemical changes in biological fluids like vitreous humour, urine, blood, cerebrospinal fluid (CSF), synovial fluid, and pericardial fluid are many. While some of the changes are the result of autolysis, the process of cells digesting themselves due to endangerment enzyme activity, others are the result of metabolism because metabolic processes continue in the tissue for variable amounts of time after death. Through the use of an innovative method we propose to refer to as "forensomics," the combined data from various methods, including thanato chemistry, could improve TSD estimation [7] [8].

1. **BRAIN (Cerebro Spinal Fluid)**

The area between the pia mater and the arachnoid mater is occupied by the cerebral spinal fluid, a clear physiological fluid. The CSF serves several purposes, including protecting the brain from pressure fluctuations, preserving a steady chemical environment, and removing waste products from cerebral metabolism. The changes which are caused due to CNS in microscopic, macroscope and chemical composition of CSF are diverse, each with pathogenicity. Some categories which are reflected are haemorrhage, infections, malignancy and demyelinating disease.[9]

Arryo et al in his study stated the phenomena where postmortem can change and alter biochemical components in body fluids like blood and CSF. Their focus was the analysis of urea, glucose, alkaline phosphatase, K-Dur 20, creatinine, cortisol, protein, and calcium in CSF fluid and compare the results between the 2 age groups with or with nonmental disease. In the outcome of their study significant differences were observed in the levels of urea between the 2 age groups. Cortisol levels revealed a significant difference between the 2 age groups[10] [11]. These include assisted transport and straightforward diffusion keep the level of glucose in the CSF constant. However, cells lining the ventricular cavities and subarachnoid spaces also use glucose, which is transported across the capillaries and arachnoid villi from the CSF. Numerous pathologic conditions can cause changes in the CSF glucose concentration. Although expectations have been reported in patients with meningoencephalitis brought on by the enteroviruses, mumps, herpes simplex, lymphocytic choriomeningites (LCM), and herpes zoster virus, levels are typically normal during viral CNS infections. In patients with severe hyperglycemia, CSF glucose concentrations rarely reach [12]. CSF is frequently tested for protein and glucose levels, cell counts and

differentials, microscopic analysis, and culture. It is also possible to do other tests such polymerase chain reaction, latex agglutination, opening pressure, and supernatant colour[13] .

Sr. No.	Composition of CSF	Biochemical Markers In CSF
1.	Electrolytes	• Potassium • Sodium • Chloride • Calcium
2.	Carbohydrates	• Glucose • Pyruvate • Lactic Acid • Inositol
3.	Non-Protein Nitrogen Compound	• Amino Acids • Ammonia • Urea • Monoamines • Creatine • Creatinine
4.	Proteins	• Total Protein • Albumin • Tan Protein • Cardiac Troponins • Myoglobin

Table 2:Biochemical markers which are found in CSF.

2. LIVER

The liver is an important organ that stores approximately 13% of the body's blood supply is always stored in the liver. There are two primary lobes in the liver. Both have 1000 lobules in each of their 8 segments. To create a common hepatic duct, these lobules are joined to small ducts that are then joined to bigger ducts. The common bile duct connects this hepatic duct to the gall bladder and duodenum, where it transports bile that has been

generated by the liver cells. The liver's primary function in the metabolism of carbohydrates is to keep the level of blood glucose stable.

Assessing liver function is crucial to evaluate overall health, and biomarkers play a vital role in the diagnostic process. The biomarkers are commonly used to determine liver function, providing a comprehensive overview of its health status are mentioned in figure 1.

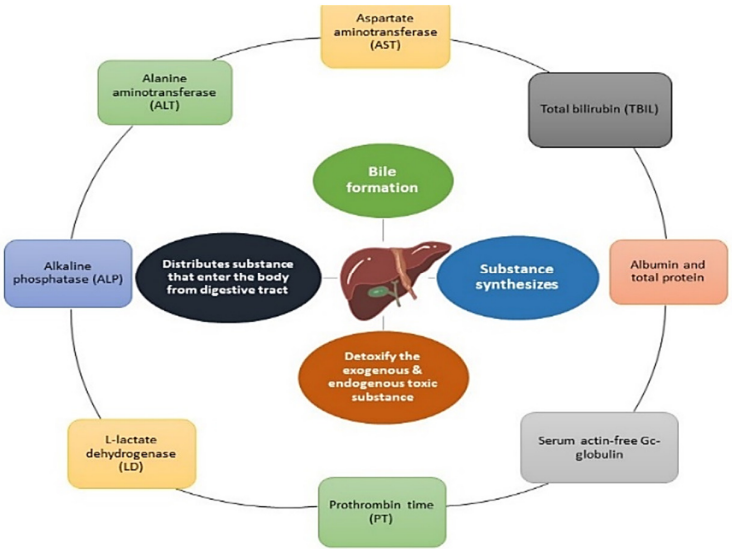

Figure 1:Liver and its vital functions: The bile in the liver synthesizes the substance, inactivates exogenous and endogenous toxicants, and distributes them all over the body.

Liver function tests offer a wealth of information in forensic investigations, providing crucial biomarkers that indicate an individual's health history and potential exposure to toxins or substances. Elevated levels of enzymes such as ALT, AST, and GGT can indicate drug or toxin exposure, which is especially relevant in cases of poisoning or substance abuse. These biomarkers are particularly useful in alcohol-related incidents, where they can indicate chronic alcohol consumption when combined with other biomarkers like bilirubin. Additionally, abnormal levels of liver biomarkers can reveal pre-existing liver conditions or diseases, providing insight into an individual's overall health. In post-mortem contexts, liver function tests can aid in estimating the time of death by reflecting changes in enzyme levels that occur during the decomposition process, while also differentiating between ante-mortem and post-mortem alterations. Finally, liver function tests are an essential component of toxicology screening, providing evidence of exposure to specific substances or drugs. However, interpreting these biomarkers requires the expertise of forensic

professionals, who must consider the broader context, including other evidence, circumstances surrounding the incident, and the individual's medical history.

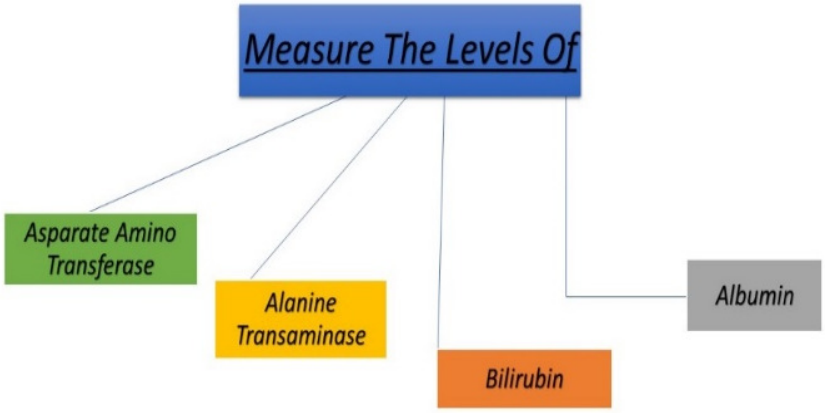

Figure 2: Measuring the levels of various markers: levels of albumin, bilirubin, alanine transaminase, asparate amino transferase will be measured in case of liver.

3.1 Albumin

The liver is where albumin is made. It has a half-life of roughly 20 days. The rate of synthesis varies with age and nutritional state. Albumin has three physiological purposes: it transports endogenous and external substances, regulates vascular osmatic pressure, and stores protein.

The body's tissues can get their amino acids from albumin. A link exists between changes in the extracellular space's colloid content and albumin synthesis. Bilirubin, fatty acids, and hormones including thyroxine, cortisol, and aldosterone are all strongly bound by it as well. Determining the blood's levels of bilirubin, proteins, and liver enzymes. Aspartate aminotransferase, Alanine transaminase, bilirubin levels, albumin, and are frequently examined [14].

3. KIDNEY

The kidneys are a pair of bean-shaped organs that reside on either side of the spinal column, below the abdominal area, and below the ribs. The purpose of the kidneys is to filter blood,

eliminate waste, regulate the fluid balance of the body, and maintain stable electrolyte levels.

The functional unit of kidney is called the nephron, which consists of proximal and distal convoluted tubules, glomerulus, Henle's loop and the collecting duct. According to National Institute of Health, the overall prevalence of chronic kidney diseases (CKD) is approximately 14% [15].

Biomarkers are essential for making an determining risk, accurate diagnosis, and choosing a course of treatment that will enhance the clinical outcome. Uric acid, Creatine, electrolytes, and urea are routinely analysed as markers of renal function, although multiple studies have established the value of markers such B trace protein, cystatin C.

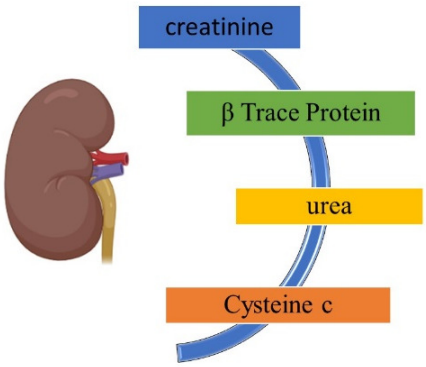

Figure 3: Kidney and the specific biomarkers: creatinine, urea cysteine C and β trace protein are the main markers which are found in kidney. Levels of these can be measured for evaluation.

Creatinine, urea, uric acid and electrolytes are markers that are found while analysing kidney. Depending on the muscle mass, the body normally produces creatinine, the breakdown product of creatine phosphate, at a fairly constant pace. Creatinine is frequently used to evaluate kidney function. The glomerular filtration rate is calculated by the national renal disease education programme using the serum creatinine concentration. Creatinine levels can fluctuate over time because a number of variables, including muscle function, muscle composition, exercise, food, and health condition, impact the generation of creatine.

- F brion et.al. In his work explained about the new technique that was employed to estimate the time of death in the weeks between post-mortems. Positively linked with post mortem estimate is the creatinine muscle concentration in human cadavers. The rate of creatinine transformation must be influenced by the

temperature. At 11 c up to 30 days and 20 c up to 15 days, there was a stronger association. The biochemical analysis which was done in this study is they collected muscle extract in 10 ml distilled water with the help of potter homogenizer. Then the sample was centrifuged, and each sample was filtered, and the creatinine concentration was determined by using an automatic analyser which is called delhomme instrumental laboratory system. They have used jaffe's method was used direct kinetics for 7s. They had confirmed results in case of human samples [16].

- Urea is major nitrogenous and product of protein and amino acid catabolism, produced by liver and liver and distributed throughout intercellular and extracellular fluid. In kidney, urea is filtered out of blood by glomeruli and partially being reabsorbed with water. Increased blood urea nitrogen (bun) is seen associated with kidney disorder or failure. High bun levels can occasionally arise in late pregnancy or because of consuming a lot of meals high in protein. If blood urea nitrogen levels are observed in fluid excess. Cristian palmiere et. Al. Stated in his paper urea nitrogen, creatinine, and acid are relatively stable in post-mortem serum and will, therefore, be used for diagnostic purposes when chronic kidney disease and end-stage kidney failure are investigated as causes of death. The outcome of their study is in urea nitrogen, creatinine, and acid concentrations in vitreous humour don't seem to be as reliable as pericardial fluid levels in estimating antemortem blood concentrations. [17]
- A protein with a low molecular weight called cysteine c is not glycosylated. Since all nucleated cells consistently produce cysteine c and that it is a known marker, entirely catabolized in the proximal tubule and freely filtered by the glomeruli. After liver transplantation, cysteine c was discovered to be a reliable indicator of glomerular filtrate in individuals with cirrhosis.
- Trace protein β since this protein is filtered out at the glomerulus and subsequently reabsorbed in the proximal tubule or discharged in urine, it may be able to meet the criteria for use as a marker of glomerulus filtration rate. It has been shown that patients with renal diseases had higher blood levels of serum trace proteins. Still a better indicator than serum trace protein is cysteine c [18].

4. HEART (Cardiac Biomarkers)

Cardiac biomarker shows up in your blood after your heart has been under severe stress and become injured because it isn't getting enough oxygen. The American College of Cardiology and the European Society of Cardiology have developed a new definition of acute myocardial infarction (AMI) that includes cardiac biomarkers [19]. cTns are

biomarkers for human ischemic heart diseases. These have been established as a biomarker of choice for monitoring the potential drug induced myocardial injury in both clinical and preclinical studies [20].

Han et al. investigated the sensitivity and specificity of cTnT in the post-mortem detection of AMI and discovered that, while cTns is a sensitive marker, it is not specific as an analytic apparatus in the diagnosis of AMI at post-mortem examination[21]. Gampon et al. used a commercial cTnT fast assay to diagnose delayed mortality from AMI in SCD patients. They discovered that femoral blood generated from fewer false positives and more accurate diagnosis. The assay proved effective in criminological practise; however, further femoral blood assays should be investigated [22].

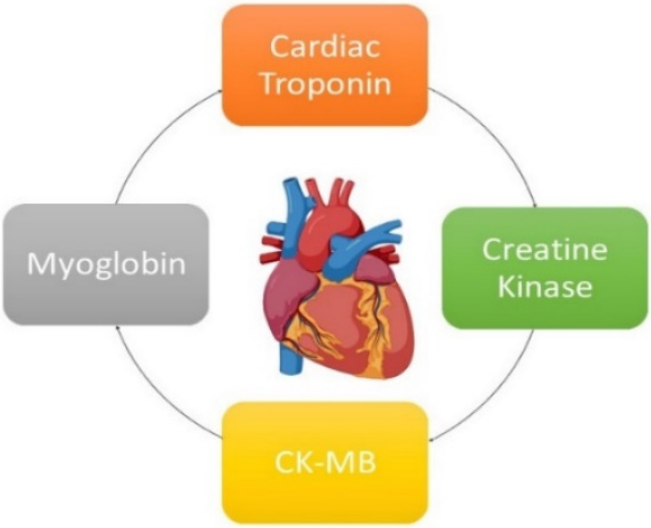

Figure 4: Biomarkers which are specified to heart: cardiac troponin, creatine kinase, CK-MB and myoglobin are the main biomarkers which are identified and measured.

The cardiac biomarkers that are diagnosed are:

- Cardiac troponin: this protein is by far the most used biomarker. It has the highest know sensitivity. It enters into your blood stream day after all other biomarkers go back to normal levels. Troponin t and troponin i are two types of troponins that can be measured. Troponin i is highly specific to the heart and stays higher for a longer period than creatinine kinase-mb. The american heart associated (aha) limits use of the other biomarkers which include ck, ck- mb and myoglobin [23]
- Creatine kinase (ck): this enzyme can also be measured several times over a 24 hrs period. It will offer at least double if you have had a heart attack. However, ck

levels can rise in a variety of circumstances besides heart attack, it's not specific. [23]

- Ck-mb: a subtype of ck. When identifying cardiac damage brought on by a heart attack, it is more sensitive. 4 to 6 hours after a heart attack, ck-mb increases. However, everything usually returns to normal within a day or two. When an expert in medicine is trying to figure out whether your recent chest pain was caused by a cardiac arrest, it is not useful. [24].
- Myoglobin: a little protein that serves as an oxygen storage. Every now and again, it is measured. Sometimes myoglobin is tested in addition to troponin to aid in the diagnosis of a heart attack. Furthermore, it is not particularly accurate in identifying a heart attack [23,24].

Alberto J. Sabucedo et.al. in his study stated that cardiac troponin I is a fundamental regulatory protein that is a component of the ternary complex that controls calcium-dependent muscle contraction. Protein is extracted from the sample, separated using denaturing gel electrophoresis (SDS-PAGE), and then visualised using a western blot with cTnI-specific monoclonal antibodies. Their approach was developed using a bovine model. The results showed bands among human cadavers, which are also known as pseudo-linear relationships between the present level of cTnI degradation and the log of the time since death, after completing all the procedures in the protocol. Additionally, a qualitative degradation band pattern that was compared to the standards was used, and the post-mortem interval could be calculated using this protocol. This method has several advantages over direct temperature methods, including a large post mortem interval, quantifiable degradation pattern, a temporal semi-quantitative relationship, and controlled temperature dependence [25].

5. BODY FLUIDS

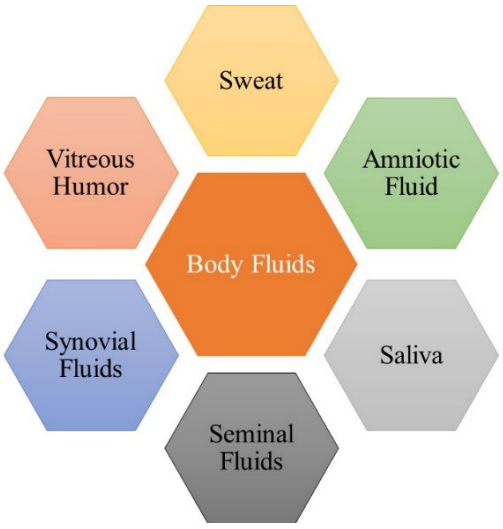

Figure 5: The biochemical markers which are present in body fluids: The body fluids which carry specific markers which can be analysed, and their levels may be measured.

5.1 Sweat: it is a fluid which is secreted by glands in the skin. Sweating's principal function is to regulate body temperature through the cooling effect of evaporating sweat. There are two types of sweat glands: eccrine glands and apocrine glands. It contains salt and urea and trace amounts of proteins and fatty acids. The biochemical test for analysis of sweat is determination of sweat chloride concentration. The suitable assay method for sweat chloride is colorimetry, coulometry and ise for sodium flame photometry or ise are suitable [9].Cristiana et al from their research stated that it is beneficial to consider sweat or urine samples for analysis of drugs rather than blood for drug treatment programs, employment initiatives and forensic investigations. The final outcome was compared and they suggest that analysis of sweat patch indicates an individual's drug use[26]. Electrochemical potentiometric sensors that rely on a heavy junction, quick response, integration, and miniaturisation are called ion selective electrons (ise). Unlike conventional liquid joint electrodes, solid ises may be employed in small, lightweight ion sensing devices. A material's hydrophobicity (water layer effect) and the interface capability (electric double-layer and/or redox capabilities) between the transducer and the ion-selective membrane are additional significant considerations. (ise) is a kind of electrochemical potentiometric sensor with integration, miniaturisation, fast response[27].

5.2 Aminotic fluid: a transparent, fluid, faintly yellowish substance that, when inside the amniotic sac, envelops the baby during pregnancy. The amniotic sac has two membranes: an exterior and an inner membrane. Throughout the course of pregnancy, the amniotic fluid's volume and chemical composition are tightly regulated, making it a dynamic medium. Polyhydramnios is the term for amniotic fluid that is produced in excess. This condition may accompany multiple pregnancy, congenital abnormalities, or gestational diabetes. One indirect way to determine the foetus's anaemia level is to do a biochemical test for bilirubin in amniotic fluids. Bilirubin at this concentration level for normal amniotic fluid is measured using scanning spectrophotometry, which scans a negative slop straight line (baseline) from 350 nm to 550 nm. If bilirubin is present the absorbance is seen maximally at 450nm[9].

5.3 Saliva: a fluid that is a combination of oral secretions from the salivary glands, cell debris, and food particles. Saliva also includes chemicals that are typically present in serum and are transported via a variety of routes, including passive diffusion.

Ultra-filtration occurs at the tight junction between cells through extracellular pathways. Saliva testing is mostly used in the fields of infections, endocrinology, and toxicology. Although these assays are not currently commercially available, saliva can be used as a non-invasive test for alcohol and drug misuse. Due to the modest amounts of analytes present, the conventional immunological tests could not be sensitive enough. The future of saliva testing might involve the use of mass spectrometry techniques for salivary proteomics[9].

5.4 **Seminal fluid**: it is a fluid which is formed at ejaculation. It is mainly composed of spermatozoa suspended in seminal plasma. The seminal plasma function as a nutrient transport medium for the spermatozoa. Some of the biochemical compounds of semen are specific to certain accessory glands and their presence or absence in the fluid can be useful diagnostically. The biochemical tests for seminal fluid are fructose, alpha-glycosidase and glycerylphoyl choline, acid phosphatase[9]. Seminal fluid is a complex solution with a combination of a huge variety of molecules mostly which are produced by sex accessory glands. Aldo et al in his review mentioned the current knowledge on the composition of semen in both vertebrates and invertebrates with internal fertilization, within the light of possible benefits of semen components of males. The role of seminal products in suppressing immune activity in female reproductive tissues in humans [20,28]**.**

5.5 **Synovial fluid**: a colorless to yellow highly viscous fluid which does not clot. Its function is to supply nutrients to cartilage, act as a lubricant to joint surface and to early away waste products. Burkhard madea et.al. Mentioned in their research paper synovial fluid is a well investigated body fluid but has very less studies in the part of medico legal investigation dealing with the concentration of alcohols, and drug distribution into synovial fluid. Their main focus was comparing values of various analytes in fluid compartments and relate them with the examination values of synovial fluid. After analyzing all the 74 cases (42 cases of sudden natural and 32 cases sudden unnatural death) they have concluded by saying that the analysis of synovial fluid are reliable on the analysis of vitreous humor, especially which it is time dependent post mortem increases of potassium concentration [29]. In case of joint diseases, an examination of synovial fluid provides vital diagnostic information. Synovial fluid analysis is used in different types of arthritis. A wide range of procedures are used to distinguish these conditions. The most commonly biochemistry test is glucose. The synovial fluid glucose content is generally no more than 0.6 mmol/l lower than the serum levels [9].

5.6 **Vitreous humor**: the vitreous humor (also referred to as vitreous fluid) may be a clear, colorless gel-like substance that fills the space within the attention between

the lens and therefore the retina. The vitreous humor is usually water with a trace of collagen, glycosaminoglycans (sugars), electrolytes (salts), and proteins. The most function of vitreous humor is to stay the attention around. The dimensions and shape of the vitreous humor also make sure that it remains attached to the retina, which is that the light-sensitive layer at the rear of the attention. The vitreous humor also can aid in the absorption of any unexpected disturbances to the eye. As there is increase in the age of an individual vitreous humor undergoes degradation forming a thinner liquid consistency, which can lead to vitreous floaters or minute disruptions in the vision like spots[30]. According to thierauf et al.'s study, pre-analytic parameters as well as analytical and instrumental variances must be considered in order to determine the detected concentrations of vitreous humour. Their study's primary focus was on the methodological examination of the two pre-treatment techniques for the samples containing variables. Centrifugation and subjecting the material to an ultrasonic water bath were the two techniques. The following parameters: creatinine, urea, lactate, potassium, chloride, and calcium. These were analysed using an ion-selective electrode or photometrically. They have determined that the results are unsatisfactory after completing the entire process. There were no notable differences between the two ocular bulbs' vitreous humours [31]. Camille et al states in their review on the most recent developments in our understanding of the metabolism of glucose in vitreous humour and, consequently, the techniques employed for the postmortem identification of diabetic challenges. Analysis and examination were performed on fructose amine, ketone bodies, lactate, and glucose levels in vitreous humor. If a close collaboration is established between pathologists and biochemists to avoid misinterpretations of the analysis performed [21,32].

6. CONCLUSION

The determination of time since death is very important for forensic experts when they are gathering evidence that can support or decline the stated actions of suspects in a crime. There are around 250 biochemical markers which are measured by various assay methodologies. Each organ in human body releases specific hormone which has its own function. After a brain examination the biomarker, called CSF, is present and is responsible for keeping the chemical environment around the brain steady, protecting the brain from abrupt pressure fluctuations, and eliminating waste products. In liver amount of albumin is estimated and its function is to maintain vascular osmotic pressure, transport endogenous and exogenous compound as a protein reserve. Kidney has four biomarkers which can be analyzed which are creatine, urea, cysteine and β- trace protein. The combined function is

to maintain the proper functionality of the organ (kidney). Heart has four markers which are cardiac troponin, creatine kinase, CK-MB, myoglobin its function is to maintain the working of heart and pumping of blood. Various body fluids also have specific markers which have specified functions like sweat, amniotic fluid, saliva, seminal fluid, synovial fluid, and vitreous humor.

In future biomarkers can be used for the identification and indication of the biological process in human body which has happened or is on-going. The concentration of chemicals or toxins can also be estimated when biomarker analysis is performed. The levels of biomarkers can change over time and various stages of the life span of one individual. Medical treatment can also affect the levels of biomarkers in the human body.

II. ACKNOWLEDGEMENT

We acknowledge ScHeme Of Developing High quality research (SHODH), Education Department, Government of Gujarat, India, for providing the research fellowship to G.V.S. We are also grateful to the Department of Biochemistry and Forensic Science, University, School of Sciences, Gujarat University by providing necessary assistance.

REFERENCES

[1] U.M. 9 M.B. Graeber, Neurogenetic diseases: molecular diagnosis and therapeutic approaches, J. Mol. Med. (1996). https://doi.org/10.1007/BF00196782.

[2] Sharona Hoffman, Cognitive Decline and the Workplace by Sharona Hoffman :: SSRN, 27 May 2022. (2022).

[3] A.E. Donaldson, I.L. Lamont, Estimation of post-mortem interval using biochemical markers, Aust. J. Forensic Sci. 46 (2014) 8–26. https://doi.org/10.1080/00450618.2013.784356.

[4] T. Henssge, C., Knight, B. H., Madea, B. and Krompecher, The estimation of the time since death in the early postmortem period (2nd Edition) -ORCA, 2017. (2017). https://doi.org/9780340719602.

[5] S.P. Michael Bohnert, , Wolfgang Weinmann, PII: S0379-0738(98)00183-2 | Elsevier Enhanced Reader, Forensic Sci. Int. (1999). https://doi.org/https://doi.org/10.1016/S0379-0738(98)00183-2.

[6] K. Strimbu, J.A. Tavel, What are Biomarkers?, (n.d.). https://doi.org/10.1097/COH.0b013e32833ed177.

[7] John Coe, Postmortem Chemistry Update, Am. J. Forensic Med. Pathol. 14 (1993) 91–117. https://doi.org/10.1097/00000433-199306000-00001.

[8] P.A. Peyron, S. Lehmann, C. Delaby, E. Baccino, C. Hirtz, Biochemical markers of time since death in cerebrospinal fluid: A first step towards “Forensomics,” Crit. Rev. Clin. Lab. Sci. 56 (2019) 274–286. https://doi.org/10.1080/10408363.2019.1619158.

[9] P.O.S. Alan Balfe, Mark Kibane, Stan Barry, Peadar McGing, Ophelia Blake, Ruth O’ Kelly, Dermot Cannon, M. Healy., The Biochemistry of Body Fluids, First Edit, Scietific Committee of the Association of Clinical Biochemists in Ireland, n.d.

[10] A. Arroyo, P. Rosel, T. Marron, Cerebrospinal fluid: postmortem biochemical study, J. Clin. Forensic Med. 12 (2005) 153–156. https://doi.org/10.1016/J.JCFM.2004.11.001.

[11] H.N. Naumann, Cerebrospinal Fluid Electrolytes after Death, Proc. Soc. Exp. Biol. Med. 98 (1958) 16–18. https://doi.org/10.3181/00379727-98-23925.

[12] P.-A. Peyron, S. Lehmann, C. Delaby, E. Baccino, C. Hirtz, Biochemical markers

of time since death in cerebrospinal fluid: A first step towards “Forensomics,” Https://Doi.Org/10.1080/10408363.2019.1619158. 56 (2019) 274–286. https://doi.org/10.1080/10408363.2019.1619158.

[13] D.A. Seehusen, M.M. Reeves, D.A. Fomin, Cerebrospinal fluid analysis, Am. Fam. Physician. 68 (2003) 1103–1108.

[14] P.L. Wolf, Biochemical diagnosis of liver disease, Indian J. Clin. Biochem. 14 (1999) 59–90. https://doi.org/10.1007/BF02869152.

[15] B.T. Kefeni, K.W. Hajito, M. Getnet, Renal Function Impairment and Associated Factors Among Adult HIV-Positive Patients Attending Antiretroviral Therapy Clinic in Mettu Karl Referral Hospital: Cross-Sectional Study., HIV. AIDS. (Auckl). 13 (2021) 631–640. https://doi.org/10.2147/HIV.S301748.

[16] F. Brion, B. Marc, F. Launay, J. Gaillledreau, M. Durigon, Postmortem interval estimation by creatinine levels in human psoas muscle, Forensic Sci. Int. 52 (1991) 113–120. https://doi.org/10.1016/0379-0738(91)90103-P.

[17] C. Palmiere, P. Mangin, Urea nitrogen, creatinine, and uric acid levels in postmortem serum, vitreous humor, and pericardial fluid, (n.d.). https://doi.org/10.1007/s00414-014-1076-z.

[18] S. Gowda, P.B. Desai, S.S. Kulkarni, V. V. Hull, A.A.K. Math, S.N. Vernekar, Markers of renal function tests, N. Am. J. Med. Sci. 2 (2010) 170.

[19] R. Jacob, M. Khan, Cardiac Biomarkers: What Is and What Can Be, Indian J. Cardiovasc. Dis. Women WINCARS. 03 (2018) 240–244. https://doi.org/10.1055/S-0039-1679104.

[20] A. Poiani, Complexity of seminal fluid: A review, Behav. Ecol. Sociobiol. 60 (2006) 289–310. https://doi.org/10.1007/S00265-006-0178-0.

[21] C. Boulagnon, R. Garnotel, P. Fornes, P. Gillery, Post-mortem biochemistry of vitreous humor and glucose metabolism: An update, Clin. Chem. Lab. Med. 49 (2011) 1265–1270. https://doi.org/10.1515/CCLM.2011.638/HTML.

[22] A. Gagajewski, M. Murakami, J. Kloss, M. Edstrom, M. Hillyer, Measurement of Chemical Analytes in Vitreous Humor: Stability and Precision Studies, ASTM Int. (2004). https://doi.org/10.1520/JFS2003152.

[23] Cardiac Biomarkers (Blood) - Health Encyclopedia - University of Rochester Medical Center, (n.d.).

https://www.urmc.rochester.edu/encyclopedia/content.aspx?contenttypeid=167&contentid=cardiac_biomarkers (accessed October 25, 2023).

[24] Chad Haldeman-Englert MD, Raymond Turley Jr PA-C, T.N.B. MSN, UC San Diego Health Health Library | San Diego Hospital, Healthcare, Healthcare. (2022). https://myhealth.ucsd.edu/SEARCH/167,cardiac_biomarkers (accessed April 22, 2024).

[25] A.J. Sabucedo, K.G. Furton, Estimation of postmortem interval using the protein marker cardiac Troponin I, Forensic Sci. Int. 134 (2003) 11–16. https://doi.org/10.1016/S0379-0738(03)00080-X.

[26] C. Gambelunghe, R. Rossi, K. Aroni, M. Bacci, A. Lazzarini, N. De Giovanni, P. Carletti, N. Fucci, Sweat testing to monitor drug exposure, Ann. Clin. Lab. Sci. 43 (2013) 22–30. https://doi.org/10.3109/10826084.2013.824477.

[27] Q. An, S. Gan, J. Xu, Y. Bao, T. Wu, H. Kong, L. Zhong, Y. Ma, Z. Song, L. Niu, A multichannel electrochemical all-solid-state wearable potentiometric sensor for real-time sweat ion monitoring, Electrochem. Commun. 107 (2019) 106553. https://doi.org/10.1016/j.elecom.2019.106553.

[28] N.J. Alexander, D.J. Anderson, Immunology of semen, Fertil. Steril. 47 (1987) 192–205. https://doi.org/10.1016/S0015-0282(16)49990-5.

[29] B. Madea, C. Kreuser, S. Banaschak, Postmortem biochemical examination of synovial fluid - A preliminary study, Forensic Sci. Int. 118 (2001) 29–35. https://doi.org/10.1016/S0379-0738(00)00372-8.

[30] E. Ankamah, J. Sebag, E. Ng, J.M. Nolan, Vitreous antioxidants, degeneration, and vitreo-retinopathy: Exploring the links, Antioxidants. 9 (2020). https://doi.org/10.3390/ANTIOX9010007.

[31] A. Thierauf, F. Musshoff, B. Madea, Post-mortem biochemical investigations of vitreous humor, Forensic Sci. Int. 192 (2009) 78–82. https://doi.org/10.1016/J.FORSCIINT.2009.08.001.

[32] A. Gagajewski, M.M. Murakami, J. Kloss, M. Edstrom, M. Hillyer, G.F. Peterson, J. Amatuzio, F.S. Apple, Measurement of Chemical Analytes in Vitreous Humor: Stability and Precision Studies, J. Forensic Sci. 49 (2004) 1–4. https://doi.org/10.1520/JFS2003152.