**BIOMARKERS**

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**Description:**

A biomarker's fundamental definition is deceptively straight forward: "A specific quality that can be assessed as a sign of healthy biological activities, pathogenic processes, or reactions to a stimulus or treatment [FDA-NIH Biomarker Working Group 2017]. Various biomarker and on the basis of alleged subtypes have been identified. Importantly, a single biomarker may satisfy several requirements for several uses, but it is crucial to establish proof for each definition. As a result, although definitions may overlap, they also have distinct characteristics that indicate specific purposes. The diagnosis and treatment of chorionic disorders depend heavily on the use of several types of biomarkers, including diagnostic, monitoring, pharmacodynamic/response, safety, predictive and prognostic biomarkers [DeFronzo and Banting lecture, 2009].

**Diagnostic biomarkers**

A diagnostic biomarker identifies a person who has a particular disease subtype or detects or verifies the presence of a disease or condition of interest. This kind of biomarker will change significantly. These biomarkers may be used to redefine the classification of the disease as well as to identify individuals who have it. For instance, the categorization of cancer detection is quickly shifting away from an organ-based classification system and toward a molecular and imaging-based classification. The evaluation of a diagnostic biomarker that has a defined context of usage and can be tested with appropriate accuracy and reliability is still difficult. Creating a validation procedure that ensures the biomarker can be assessed accurately, repeatedly, and at a cheap cost is one objective. Assays are frequently not validated, lead to inaccurate perceptions of the value of the biomarker [Wang et al., 2005].

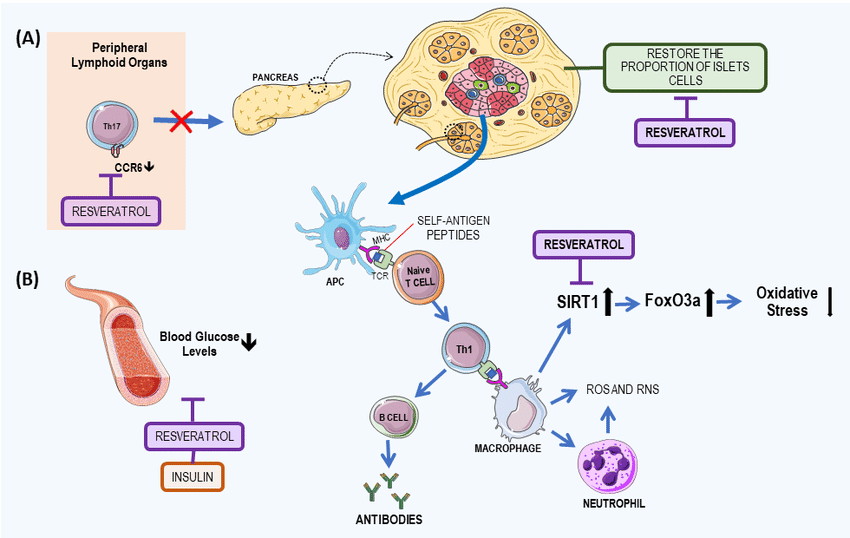
**DIABETES**

**HISTORY OF DIABETES MELLITUS**

Type-1 diabetes mellitus [T1DM] is an endocrine disorder. According to the writings published by Harold Himsworth’s son Richard in 1936 about diabetic medicine, two types of diabetes were distinguished. Harold Himsworth defined them as ‘insulin – insensitive’ and insulin – sensitive. These classifications are today commonly referred as ‘type 1’ and ‘type 2’ diabetes.

**INTRODUCTION**

The endocrine disorder known as type 1 diabetes mellitus (T1DM) causes the pancreatic cells to stop producing insulin, usually as a result of autoimmune destruction. This results in hyper glycemia and ketosis; thus, insulin replacement is vital to management. The prevalence has increased during puberty and the early years of adulthood, but onset can happen at any age. From last decade onwards persons with T1DM are highest among adults. Symptoms include polyuria, polydipsia, and weight loss. Diabetes ketoacidosis is one of the acute complications that needs immediate attention. Long-term complications include microvascular and macrovascular disease. Other autoimmune diseases and psychosocial problems are more frequently occur in T1DM patients. Optimizing glucose control should be the main management goal to minimize both short-term and long-term complications.



Diabetes type 1, also referred to as infantile diabetes or insulin-dependent diabetes, is a chronic illness. Diminutive to no insulin is produced by the pancreas in this condition. The body uses the hormone insulin to allow glucose (sugar) into cells so those cells can produce energy. Type 1 diabetes may be brought on by a variety of factors, including genetics and certain viruses. [Nerup et al., 1974]. Despite typically developing in childhood or adolescence, type 1 diabetes can also strike adults. Type 1 diabetes still has no cure, despite extensive research. The goal of treatment is to control blood sugar levels with the help of insulin, diet, and lifestyle changes in order to avoid complications [Kolb, 2005].

**The protagonist of insulin**

When a significant amount of islet cells is destroyed, the body produces little or no insulin.

Insulin is a hormone that secreted from a gland late and beneath the stomach (pancreas).

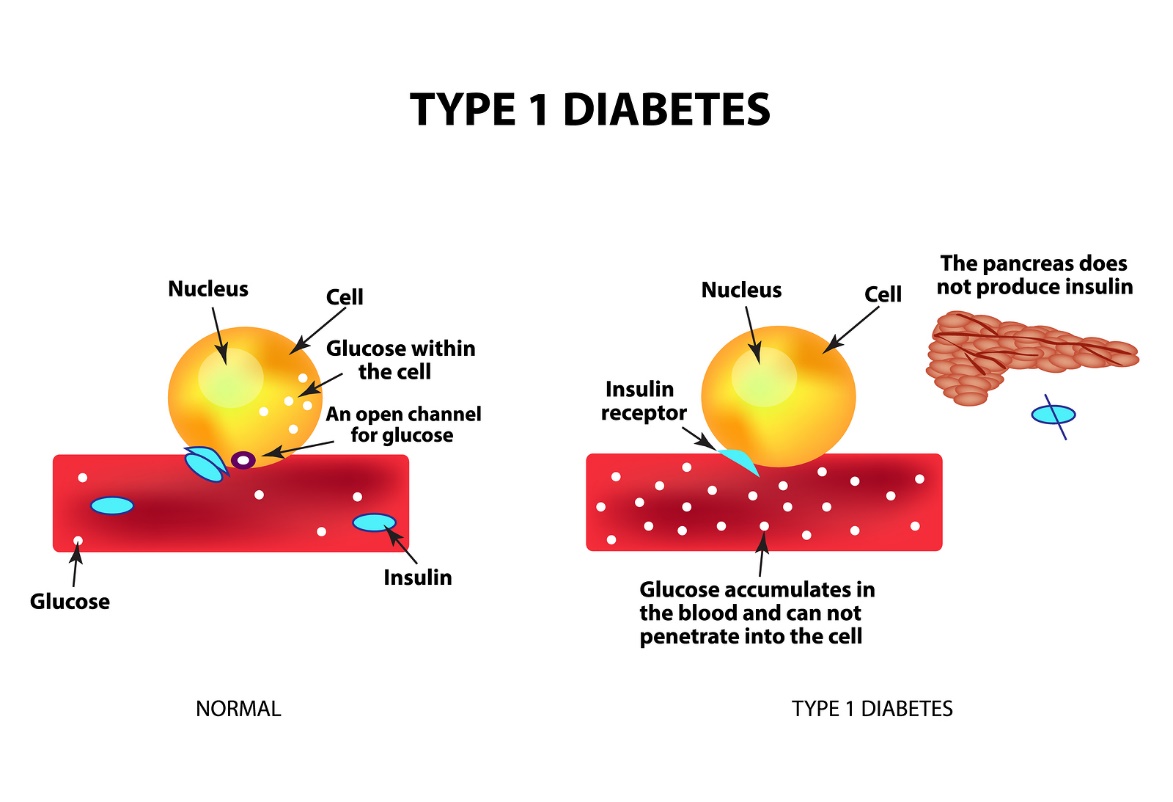
* Insulin travels through the bloodstream the from the pancreas.
* Throughout the body, insulin moves, enabling sugar to enter the cells.
* The amount of sugar in the blood is decreased by insulin.
* The pancreas produces less insulin as the blood sugar level rises.

**The function of glucose**

Glucose — a sugar — is a main source of energy for the cells that make up muscles and other tissues.

* Food and the liver are the two main sources of glucose.
* Sugar is absorbed into the bloodstream, where it crosses the threshold cells with the help of insulin.
* Glycogen, a form of glucose, stores in the liver.
* The liver converts stored glycogen into glucose when blood glucose levels because they are low, when you haven't eaten for a while. As a result, glucose levels stay within the normal range.

There is no insulin to allow glucose into the cells in type 1 diabetes. Sugar accumulates in the blood as a result. Life-threatening complications may result from this. Complications from type 1 diabetes over time may have an impact on the body's major organs. The heart, blood vessels, nerves, eyes, and kidneys are some of these organs. The risk of numerous complications can be reduced by maintaining normal blood sugar levels.



Diabetes complications can lead to disabilities or even threaten your life.

* **Blood vessel and heart disease.** Diabetes reduces the risk of some heart and blood vessel problems. These include hypertension, heart attack, strokes, coronary artery disease with chest pain (angina), and artery narrowing (atherosclerosis).
* **Nerve damage (neuropathy).** If there is too much sugar in the blood, the walls of the tiny blood vessels (capillaries) that supply the nerves may be harmed. Tingling, numbness, burning, or pain may result from this. This typically spreads upward from the tips of the toes or fingers. Your affected limbs may gradually lose all feeling as a result of a blood sugar low. Problems with nausea, vomiting, diarrhea, or constipation can result from damage to the nerves that control the digestive system. Erectile dysfunction may be a problem for men.
* **Kidney damage (nephropathy).** The kidneys' countless tiny blood vessels stop waste from entering the bloodstream. Diabetes could harm this system. Severe damage can lead to kidney failure or chronic end-stage kidney disease. Dialysis or kidney transplantation are the only treatments available for end-stage kidney disease.
* **Eye damage.** Diabetes (diabetic retinopathy) can impairment the blood vessels in the retina, the area of the eye that detects light. Blindness could result from this. Diabetes as well raises the risk of glaucoma and cataracts, two serious eye diseases.
* **Foot damage.** If there is nerve damage in the feet or poor blood flow to the feet, specific foot complications are more likely to develop. Blisters and cuts can develop into dangerous infections if left untreated. Amputation of the leg, foot, or toe may be essential for the treatment of these infections.
* **Skin and mouth conditions.** You could be more vulnerable to skin problems and mouth infections if you have diabetes. Infections triggered by bacteria and fungi are among them. Dry mouth and gum disease are also more probable.
* **Pregnancy complications.** Both parents and babies may be in danger from high blood sugar levels. When diabetes is poorly managed, the risk of miscarriage, stillbirth, and birth defects rises. Diabetes increases the parent's risk of developing preeclampsia, diabetic ketoacidosis, retinopathy, high blood pressure brought on by pregnancy, and diabetic ketoacidosis.

**SERUM/AUTOANTIBODIES BASED BIOMARKERS**

Risk factors from both parents may be inherited in the majority of type 1 diabetes cases. It first embodies in early childhood or adolescence. Adults can develop it. There is still no diagnosis for [T1DM], only early prediction may help to control the food and lifestyle to maintain a good health. T1DM show symptoms only at later stage of 12-14 years age people. For the diagnosis of T1DM, serum biomarkers which includes the combination of glucose, glycated molecules, c-peptide and autoantibodies. In this case study we diagnosed T1DM using GADA [Glutamic and decarboxylase AAb], ICA [Islet cell cytoplasmic AAb] and IAA [Insulin Autoantibody].

In any case one of these 3 biomarkers present in more than 95 percent of T1DM patients upon Hyper glycemia detection. GADA helps in the test for AAb against B-cell protein [antigen]. It is not specific to beta cells. Diabetic patients with the presence of GADA indicates the presence of autoimmune destruction. Approximately 70-80 percent of persons with new -onset type 1 diabetes have the presence of GADA; it indicates the increased risk for the progress of T1DM. ICA involves in the measurement of a group of islet cell AAb targeted against islet cell protein whereas beta cells are also types of islet cells. Like GADA, the presence of ICA indicates the presence of autoimmune destruction, ICA is present in 70-80 percent of new onset type 1diabetic patients. IAA is targeted to insulin [autoantibody against antigen]. The only antigen thought to be highly specific for beta cell is insulin. Patients with diabetes with the presence of IAA indicates the increased risk of Type 1 diabetes. It is confirmed strictly when patients weren't taking insulin treated for 14 days before IAA testing. This AAb are present in 50 percent of children with new onset of type 1diabetes and uncommon in adults. Autoantibodies that target the endogenous insulin and autoantibody produced against exogenous insulin are not distinguished by IAA test.

**TYPE -2 DIABETES MELLITUS**

Type 2 Diabetes Mellitus (T2DM) is a chorionic disease, contemplated to be foremost global health issue. Within the past 35 years, it is fourfold from 108 million to 422 million in 2014 and the expected level in 2030 is 552 million [William, 2008]. T2DM is distinguished by dysregulation of carbohydrate, lipid and protein metabolism, which leads to impaired insulin secretion and insulin resistance, sometimes both of these. Among the three major types of Diabetes, T2DM is further communal than Type 1DM or Gestational diabetes.

T2DM is a complex illness caused by hereditary and environmental factors and the changes are specified by B -cell dysfunction, insulin resistance and chorionic inflammation. Type 2 Diabetes Mellitus does not ensue unless the B -cells are unable to produce sufficient amounts of insulin to even out the insulin resistance. Factors that contribute to B -cell failure are genetic abnormalities, ageing, incretin hormone resistance or deficiency [gastric inhibitory peptide (GIP) and glucagon like peptide 1(GLPP)], glucotoxicity, lipotoxicity. Insulin resistance results to B -cell stress, reactive oxygen stress, hypersecretion of islet amyloid polypeptide IAPP and activate the inflammatory pathways such as CRP, PAI 1, IL-6, IL-18. DeFronzo and Banting lecture (2009).

**CRP**

The occurrence of congestive heart failure, cardiomyopathy and CHD is more in patients of type 2 diabetes mellitus. The mortality rate of CHD in patients of type 2 diabetes mellitus is two times more in males and four times more in females according to Framingham heart study. As diabetics are more at risk for CHD so there is a need to search out for new biomarkers which would help to evaluate the risk for development of CHD in these patients. Elevated level of CRP is a better and strong indicator of CHD in type 2 DM than any other risk markers due to its basic role in atherosclerosis [Lyer and Desai, 2010]. It enhances the release of tissue factor from macrophages, leads to activate the complement system and causes the aggregation of LDL-C and VLDL-C by binding with them18-20. Due to involvement of inflammatory mechanisms in diabetogenesis and atherosclerosis, CRP levels tend to be increased in patients of type 2 diabetes mellitus with CHD21,22. Our results show high levels of hs-CRP in patients having type 2 diabetes mellitus with CHD as compared to patients having type 2 diabetes mellitus without CHD [Lyer and Desai, 2010]. These results are in tune with the reports presented by Mohan et al., (2005) and Leipold et al., (2005). A meta-analysis of 2000 studies, comprised of about 1953 coronary accidents also showed that a single initial base line CRP value in upper third suggests the risk of 2.0 for future coronary accident as compared to a CRP value in lower third of the distribution, as seen in general population [Rafique et al., 2006]. These findings concurred with those of normal subjects with the diabetic population and found no significant difference in CRP values [East, 2006]. The positive association of hs-CRP with FBS and HbAlc can be explicated by the datum that inflammation is the main hallmark state of insulin resistance which is observed in obesity and type 2 diabetes mellitus [Roberto, 2006]. Inflammation can be caused by two different mechanisms. First, long-term overeating and intake of glucose cause an oxidative stress and pro-inflammatory state; second, the release of TNF- and IL-6 inhibits the action of insulin by blocking the transduction of insulin signals 34, 35. As per the study, elevated plasma hs-CRP levels in type 2 diabetic patients may contribute to ongoing atherosclerotic processes that result in the development of coronary heart disease in these patients. Patients with diabetes mellitus may be able to use this as a marker for the development of atherosclerosis [Pepys, 2003].

**IL-6 AND IL-18**

IL-6 is a cytokine which acts in both the innate and adaptive immune response. IL-6 is synthesized by monocytes, fibroblasts, endothelial cells or other cells in response to the other cytokines stimulation. IL-18 is a cytokine from IL-1 family. It has a pleiotropic action and it participates in both the innate and in acquired immune response [Shoelson, 2006]. Hyperglycemia is a characteristic of glucose intolerance. IL-6 and IL-18 shows serum level variations which are positively correlated i.e, more significant increases in hyperglycemia spikes. Hyperglycemia is a common situation in diabetic patients [Bastard, 2006].

Atherosclerosis initiates a very slight increase in CRP, so measuring it requires the use of extremely sensitive techniques. Acute myocardial infarction (AMI) risk is emerging in associated with CRP has persisted in the Honolulu Heart Program even after 20 years. Hyperglycemia itself, a characteristic of glucose intolerance, is related to the immediate synthesis of markers such as IL-6 and IL-18, with serum level variations positively correlated and with more significant increases in hyperglycemic spikes, a situation that is common in diabetic patients [Sjohoim, 2005].Since patients with diabetes comprise a significant part of the people with coronary artery disease (20-24%), the understanding of the inflammatory mechanisms in diabetes and also in insulin resistance is fundamental for a proper treatment [Kannel, 1978].

**Methods**

The patients could be utilized, enrolled, and given a conversant harmony method previously having their veins sampled for IL-6, IL-18, high-sensitivity C-reactive protein, and markers of myocardial necrosis. Blood was also used for determinations of blood count, coagulation tests, urea, creatinine, sodium, potassium, blood glucose, and lipid profile [King and Aubert, 1998]. No additional venous puncture was performed in unstable patients, since punctures are performed as a routine diagnostic procedure. All patients received the usual treatment and no medication was added or discontinued by study intervention [Roberto, 2006]. Patients who were unbalanced were closely watched in the coronary unit or emergency room. Patients had the option to leave the study whenever they wanted. The samples were centrifuged and kept at -80°C for further analysis. The mean time elapsed from the last episode of pain and blood collection was 14 hours. Serum levels of IL-6 and IL-18 were determined using the ELISA technique, with the R&D Systems (Minneapolis, MN, USA) and MBL-Medical & Biological Laboratories (Nagoya, Japan) commercial kits, respectively, according to the manufacturers. A distinctive monoclonal antibody is adsorbed onto a plate using this procedure. After addition of the serum sample where the mediator to be determined is placed, the material is incubated, and this is the moment when the antigen molecules will bind to the antibodies adsorbed onto the plate. All unbound material is washed away. Then, using the sandwich technique, a fresh antibody that is specific for an antigenic determinant bound to the plate is added. To get rid of the unbound antibodies, the material is washed once more. Then, in proportion to the amount of the mediator present in the sample (antigen), a substrate that has the property of changing color when in contact with the enzyme is added. The reading is performed in a plate reader (BioRad, Tokyo, Japan) at 450 nm and in comparison, to a standard curve produced using known amounts of the recombinant mediators. For IL-6, the detection limits for the essays were 0.09 pg/ml, and for IL-18, they were 12.5 pg/ml. The change in serum levels of IL-6 and IL-18 shows the best results in the diagnosis of Type 2 Diabetes Mellitus [Pradhan et al., 2003].

**OBESITY**

Nearly all nations in the globe are experiencing an upregulation in the obesity epidemic, and future rises are anticipated [Sakkinen, 2002]. Men in Western high-income countries and women in Central Asia, the Middle East, and North Africa have the highest obesity prevalence rates. Obesity is linked to a shorter life expectancy and a range of chronic disorders, most notably type 2 diabetes, coronary heart disease, and some types of cancer [Pradhan, 2001]. As a result, obesity is one of the most significant public health issues of our time and has substantial allegations used for together the healthcare system and individual health. Despite the fact that research have shown that accounting for body fat distribution with measurements like waist circumference may improve disease prediction, obesity is traditionally defined based on body mass index (BMI), contempt the fact that the BMI is known to be an imperfect measure of excessive or abnormal body fat accumulation [Shoelson et al., 2006]. Analyzing the molecular processes that cause the correlation between obesity and chronic disease has identified a number of biomarkers as potential mediators. These biomarkers for obesity include circulating cytokines and hormones like adipokines, which are hormones secreted by adipose tissue, as well as indicators at other biological levels like genetic or transcriptome markers that have recently been developed by more advanced omics technologies. The probable for using obesity biomarkers for a different or expanded more accurate characterisation of the obese phenotype that is important for disease exists.

Insulin resistance, or reduced insulin-mediated glucose absorption, has been linked to obesity for more than three years [Haffner, 2006]. Long-term insulin resistance is linked to hyperinsulinemia, which is hypothesized to result from the pancreatic beta cells' compensatory increase in insulin secretion to lower the raised blood glucose levels [Sjohoim and Nystrom et al., 2005]. A distinct series of events has recently been proposed, according to which obesity first causes hyperinsulinemia, which is then followed by insulin resistance brought on by downstream pathways [Kannel et al., 1979]. Obesity and chronic diseases like diabetes and cardiovascular disease may be related through insulin resistance and hyperinsulinemia. The IGF system, an evolutionary conserved set of factors that has an ongoing impact on growth, is strictly connected to insulin metabolism [Garcia et al., 1974]. IGF-1 has been linked to the formation of cancer because it affects cell proliferation, differentiation, migration, and survival in both healthy and genetically damaged cells [Susan et al., 2004]. Additionally, it has been established that insulin has growth-promoting effects by boosting cell proliferation and suppressing apoptosis [Ridker et al., 2002]. As a result, insulin may have an impact on cancer risk. By downregulating the synthesis of IGF binding proteins (IGFBP-1, IGFBP-2) on the one hand and by increasing hepatic IGF-1 production on the other, hyperinsulinemia increases the bioavailability of free, active IGF-1. Thus, one molecular mechanism between obesity and the risk of developing cancer has been suggested using insulin and the IGF axis [Cermak et al., 1993]. Contrarily, experimental trials on public with diabetes revealed that administering IGF-1, particularly in conjunction with IGFBP-3, lowers the need for insulin and enhances glucose homeostasis [Pradhan et al., 2001]. Additionally, according to experimental studies, IGF-1 lowers the burden of atherosclerotic plaque through controlling oxidative and inflammatory processes, cell senescence, and epigenetic alterations [Dutor, 1997]. Adipokines are a group of hormones secreted by the adipose tissue, an active endocrine organ that mediates the metabolic and inflammatory effects of obesity and may establish a relationship between obesity and disease risk Leipold, [Haffner, 2006]. Leptin and adiponectin are the most prevalent and best understood adipokines, whereas more recently, adipokines like resist in, fatty-acid binding protein-4 (FABP-4), omentin, lipokalin-2, and chemerin have been suggested to play a role in the negative health effects of obesity. Adipose tissue is where adiponectin and leptin are largely expressed. In divergence to the majority of other adipokines, adiponectin expression is downregulated in adipose tissue in obese persons, leading to the finding that obese people have lower levels of adiponectin than people of normal weight. In accumulation of its role in energy metabolism, adiponectin also has anti-inflammatory and insulin-sensitizing properties [Danesh et al., 2002]. A meta-analysis of 16 prospective cohort studies found no evidence of a linkage among circulating adiponectin and coronary heart disease or stroke [Dutor, 1997], despite the fact that adiponectin has been suggested to have cardioprotective and anti-atherogenic properties. The idea that adiponectin was also disproven to play a causal role in the pathophysiology of coronary heart disease by a Mendelian Randomization study [Brownlee., 2001]. Adiponectin has been linked to a protective role in the development of cancer, especially colorectal cancer, either directly through its role in the inhibition of cell growth and induction of apoptosis or indirectly complete its positive effects on insulin sensitivity and decreased inflammation [Nimptsch, 2017]. A link between circulating adiponectin levels and a reduced risk of colorectal cancer has been found [Cancer Prev Res (Phila)], although a subsequent Mendelian Randomization research found no evidence of a causative relationship [Pischon, 2009]. As an adipokine that measures adipose tissue mass, leptin is higher in obese people than in those of normal weight [Mohan et al., 2010], suggesting that there is a leptin-resistant state in obesity. According to Pischon (2009), leptin's primary role is to regulate energy balance and hunger over the long term. When this function is compromised by leptin resistance, obesity results. Owing to its reputation as a pro-inflammatory adipokine that corresponds with cardiovascular risk factors including hypertension, leptin has been proposed to mediate the upregulated risk of cardiovascular illnesses linked with obesity [Haffner, 2006].

**ALZHEIMER’S DISEASE**

At present, 48 million people worldwide are suffering from dementia and this number is projected to increase to 131 million by 2050 [Alzheimer’s Association, 2010]. The most frequent form of dementia is alzheimer's disease (AD), which is clinically characterized by a progression from memory problems that occur only occasionally to a slow overall decline in cognitive function. Dementia was expected to impact 44 million people worldwide in 2013 [Santana et al., 2015]. The biggest clinical need in neurology is still Alzheimer's disease because there are currently no therapies with demonstrated disease-modifying effects. The pathology of AD is characterized by a complex interplay of several biochemical changes, such as modifications in the metabolism of amyloid precursor proteins, phosphorylation of the tau protein, oxidative stress, diminished energetics, mitochondrial dysfunction, inflammation, membrane lipid dysregulation, and disruption of neurotransmitter pathways. The majority of these clinical characteristics are distinctly related to anomalies in metabolism. Metabolic dysfunction is progressively documented as a significant contributor to AD. For instance, decreased cerebral glucose absorption is a constant aspect of AD and manifests decades before cognitive loss does. Because of the interaction between A42 and mitochondrial enzymes, which increases the release of reactive oxygen species (ROS), glycolysis, the TCA cycle, and mitochondrial respiratory-chain activity are all impacted, as well as the accumulation of harmful intermediate metabolites in the mitochondria, by the well-documented neurotoxicity associated with A42.

**Alois Alzheimer and Auguste D**

The dementing syndrome that would eventually be known as AD was initially described by the German neuropathologist and psychiatrist Dr. Alois Alzheimer. Alzheimer discussed in his influential 1906 conference lecture and a subsequent 1907 article, Auguste D. discussed the case of a 51-year-old woman with a "peculiar disease of the cerebral cortex." Progressive memory and language loss, disorientation, and behavioral symptoms (hallucinations, delusions, paranoia) and psychosocial impairment were all present in Auguste D [Chiang, 2014].

**Normal memory**

To comprehend the complexity of dementia, it is vital to define what happens during normal ageing as well as comprehend what might go wrong and give rise to aberrant conditions such as dementia. Ageing can be classified into biological, social, and psychological fields, but there is frequently significant overlap and interaction between them. A bodily change, such as arthritis, can, for example, decrease mobility, which can reduce participation in social activities or other previous sources of satisfaction. The impact of one component of ageing on another should also be considered; this is critical when considering and contrasting historical and present cognitive functions in the same person. It is difficult to define 'normal,' and it is astonishing how 'normal' and 'abnormal' behaviours and attitudes sometimes coincide. Boundaries are blurred across different cultures, settings, and even between individuals. A common misunderstanding is that normalcy is separate from abnormally, whereas in fact 'normally' refers to the 'range around the middle of a dimension (eg height) with two extremes at opposing ends (extremely tall and very low), rather than one extreme. varied people have varied ideas about what constitutes normally, hence people's expectations about ageing differ. Individuals are generally living longer lives as medicine and technology develop, thus more individuals are exposed to older people and experience the varying ages of relatives and acquaintances [Povova et al., 2012]. As a result, people's perceptions of normal ageing are continually evolving, as are their expectations of themselves and others. Normal ageing causes changes in an individual's appearance, however modest, as well as specific alterations in higher brain functions or 'cognitive' functions. Memory can also be harmed. Sometimes this is because the individual did not absorb the information correctly, and other times it is because it can no longer be efficiently encoded or preserved. The impact of ageing on memory, specifically episodic memory is frequently one of the first cognitive functions to be seen by others and can cause significant distress to the individual as well as relatives, close friends, and careers. Deterioration in memory functioning is a hallmark of dementia. However, it can also signal other dysfunctions, which should always be taken into account in any examination [Blennow et al., 2006]

**Modes:**

There are three main stages to the disease, each with their own challenges and symptoms. Medical experts can forecast future symptoms and potential therapeutic options by determining the disease's stage at the moment. The symptoms of AD vary in severity and are specific to each individual case. Inheritance of certain genes is a risk factor for AD, with both familiar and sporadic cases occurring. In sporadic AD, which is the more common form, there is a link with the apolipoprotein 4 (APOE4) allele, with the risk being greater in homozygotic situations [Forstl and Kurz, 1999]. Alzheimer's disease is influenced by vascular factors, psychological factors, and environmental factors. The disease has three main stages, each with a unique set of symptoms. It has its own set of problems and symptoms. Physicians can determine what symptoms to expect by determining the disease's current stage. be predicted in the future and alternative courses of treatment. Each case of Alzheimer’s disease has an own collection of symptoms that vary in severity [Chou, 2014]. Certain gene inheritance is a risk factor for Alzheimer’s disease, with both familiar and sporadic cases occurring. The apolipoprotein 4 (APOE4) allele has been linked to sporadic Alzheimer’s disease (the more prevalent variety), with the risk being higher in homozygotic conditions. Alzheimer's disease is caused by a combination of environmental, vascular, and psychological factors. There are currently no drugs that can stop the progression of neurodegeneration in Alzheimer's disease; instead, symptomatic care is provided. In mild to severe Alzheimer’s disease, for example, cholinesterase inhibitors (CIs) that increase cholinergic neurotransmission are utilized. Memantine, a N-methyl-D-aspartate (NMDA) receptor antagonist, is used to avoid excitotoxicity in mild to severe cases, and antipsychotics and antidepressants are used to treat neuropsychiatric symptoms. There is currently no known way to prevent Alzheimer’s disease. Prevention measures are being researched and developed on a daily basis. So far, the strongest data suggests that you may be able to reduce your risk of Alzheimer’s disease by lowering your risk of heart disease. Alzheimer's disease and vascular dementia are both risks of heart disease that are largely increased by the same risk factors. High blood pressure, high cholesterol, being overweight, and diabetes are all important risk factors. Alzheimer's disease is complicated, and no one drug or other intervention is likely to result in effective treatment [Galasko, 1998]. Current treatments are aimed at assisting patients in maintaining mental function, managing behaviour problems, and slowing or delaying illness signs. Researchers want to create medicines that target specific genetic, molecular, and cellular systems in order to stop or prevent the fundamental cause of the disease.

**RISK FACTORS**

**Age:**

One of the non-modifiable risk variables is age, which is the single maximum risk factor for acquiring Alzheimer’s disease. The majority of Alzheimer’s disease instances are seen in those over the age of 65. Alzheimer's disease affects about 5% of persons between the ages of 65 and 74. The risk rises to 50% for people above the age of 85. Numerous studies have initiated that ageing can affect the body's self-repair processes, including those in the brain. Furthermore, several cardiovascular risk factors, such as high blood pressure, heart disease, and excessive cholesterol, worsen with age [Emilien, et al., 2004].

**Genetics:**

There is no suggestion of a genetic pattern of inheritance in sporadic Alzheimer’s disease. The gene for apo lipoprotein E (ApoE) has been linked to the onset of Alzheimer's disease. This gene is thought to be in charge of the protein that transports cholesterol in blood vessels. ApoE4 has been demonstrated to raise the likelihood of developing the condition to a larger level. The ApoE2 variant, on the other hand, protects against the disease. A chromosomal mutation may be to blame in cases occurring before the age of 65. This uncommon form of the disease is known as Familiar Alzheimer’s disease, and it affects less than 10% of Alzheimer’s sufferers. Mutations on chromosomes 1, 14, and 21 have been found to be the cause. If a single chromosomal mutation is inherited, the person is 50% more likely to acquire Alzheimer’s disease. In many cases, the prevalence and incidence of Alzheimer’s disease strongly showed that age is the most relevant known risk factor. Indeed, AD prevalence rises dramatically with age, with (AD) incidence rising from 2.8 per 1000 person years in people aged 65 to 69 to 56.1 per 1000 person years in people aged 90 and later.

**Coexisting Health Problems:**

It has been discovered that there is a substantial correlation between an Alzheimer’s patient's cardiovascular health and brain health. Having heart disease, high blood pressure, or both Certain or high cholesterol levels can raise the likelihood of developing Alzheimer’s disease. This is caused by damage to blood arteries in the brain, which results in decreased blood flow and possibly brain tissue loss. Diabetes type 2 may potentially increase the risk of Alzheimer’s disease. Inadequate insulin conversion to energy may result in greater amounts of sugar in the brain, causing significant injury to the entire body [Mellon et al., 2009]. As in almost every case, symptoms such as amnesia and confusion are moderate in the early stages of the disease, but they steadily deteriorate as the disease continues and brain damage becomes more severe and evident. Some persons with Alzheimer’s disease also suffer from acute depression and are unsure how to cope with the loss of cognitive and fundamental functioning. Among the symptoms of depression are:

•Insomnia

•Mood swings

•Less contact with the people around

•Difficulty in concentrating

**Diagnosis;**

Diagnosis Criteria: There is a logical progression for the clinical diagnosis of Alzheimer's disease as is observed in many diseases: the history should include information from an informantion i.e., the person related to the patient, a mental state assessment should include a validated cognitive function test; and the physical examination should focus on vascular and neurological signs supplemented by investigations and patient history [Lukiw, 2012]. Assessment of dementia involves a two-step process in most cases. Firstly, it is important to distinguish dementia syndromes from other conditions that can mimic them, such as depression, delirium, and mild cognitive impairment as is observed in most cases, therefore these diseases need to be distinguished first. Secondly, once dementia syndrome is recognized, the diagnosis of a subtype is important because it may determine the kind of treatment possible. Due to its non-confrontational nature and the fact that a normal drawing of a clock more or less rules out the presence of significant cognitive impairment, the clock test is widely used for cognitive screening in general practice. However, using a single cognitive test to check for the presence of dementia syndrome can be challenging due to the rules for scoring the tests, which can be quite complicated. does not do justice to the wide variety of symptoms and indications that make up the clinical syndrome of dementia. Activities of daily living are assessed alongside cognition, but there is less consistency in the assessment instruments used [Thies and Bleiler, 2013]

Detection Methods: Neuroimaging is a promising and rapidly growing field of study for detecting Alzheimer’s disease. There are a variety of brain imaging technologies that can be used to detect abnormalities in the brain, including PET, MRI, and CT scans, which are considered preliminary testing for illness identification [Francis et al., 1999]. Each scan employs a distinct technique to discover specific structures and abnormalities in the brain and its connected regions. Although brain imaging is not now a common aspect of Alzheimer’s disease testing, recent clinical studies have showed encouraging results that may transform how physicians diagnose the condition. Despite many years of dedicated and productive study, no effective medication for Alzheimer’s disease, the most common form of dementia, currently exists. has evolved. It has become increasingly clear that, if the disease is to be treated successfully, it must be detected as early as possible, perhaps even before symptoms are evident. Thus, there is a great need for reliable diagnostic methods so that treatment to slow or prevent the disease can begin as early as possible to treat the disease in proper way [Corbett et al., 2013].

The typical pathological feature of Alzheimer's disease is formation of insoluble amyloid plaques in the brain and nerve cells. These panels may exist measured in the brain using positron emission tomography (PET camera) to visualize the binding of radioactive tracer particles amyloid plaques. It also measures amyloid levels in the spinal cord [Thies and Bleiler, 2013]. Despite the accumulation of amyloid in the brain. Alzheimer's disease, study shows amyloid levels. Instead, atrophy of the spinal cord. Check out this study. These men compared PET measurements of amyloid in the brainiest beta amyloid 42 in the cerebrospinal fluid to see how they line up this. The research was carried out in seven European memory clinics. 230patients were tested for memory problems and dementia The patient received many different diagnoses, including benign one’s Cognitive impairment (CLD), Alzheimer's disease, etc. type of dementia PET: Positron emission tomography (PET) uses radiation signals. At the end, create a colorful 3D portrait of a person body. Patients receive radioactive injections, which includes radioactive materials with natural chemicals This chemical is normal in the California Alzheimer's study Glucose is widely used. The radiation reaches the organs. This molecule is used as energy. From the settings metabolism releases positrons, the energy of these places a PET scan detects an itron and converts the input into images. The resulting screen image. This image shows the function of the patient's body, showing how effectively the radiotracer is being used broke up the amount of emitted positron energy generates different colors and intensities that reflect the range of brain function a PET scan can detect changes metabolism, blood circulation and cellular communication processes in the brain and other brain activities. The study was published in 1996 in the Journal of Clinical Psychiatry described a method for using PET scanning to detect changes in glucose metabolism in the Alzheimer's disease brain the patient It was found that in parietal, temporal and pos upper cortices, glucose metabolism is unusually low seen Their number was further reduced in patients with the disease is advanced and affects more places brain. Small and colleagues found that PET the scan can be used to detect changes in glucose metabolism long before the clinical manifestation of symptoms. In addition, A PET image could also be used for diagnosis the effectiveness of the treatment of Alzheimer's disease [Beyenhof and Lauren, 2010]. CT: Computed tomography (CT) makes a series of CT images Sectional view of the body. using a computer, Individual scans are combined and merged into one detailed image. Scanners provide information to doctors Body density and different tissues parts of the brain Contrasting colors can be used to improve clarity injection to isolate homologous tissues [Khachaturian and Zaven, 1999]. MRI: The first magnetic resonance imaging technology 1977 It was used to create 2D or 3D images. Effects on the body and can be used to diagnose injuries and diseases. East- A superconductor is an important component of the MR system magnets produce a large and stable magnetic field. Smaller gradient magnets produce weaker magnets domain here. These magnets can make different parts of the body. Scanner The human body is made up of billions of atoms. But the hydrogen atom becomes magnetic in the field of networking. Each hydrogen atom is rotated randomly hub, but the particles in the MR field corresponds to the direction of the field. half an atom Point to the patient's head, halfway to the feet, they cancel each other out. How many atoms per million It has not been cancelled. Then the machine emits radio frequencies The intrinsic moment of hydrogen that spins these protons otherwise. When the spin stops, the proton. It releases the energy that the system interprets reverse each type of fabric responds and appears differently to Trast dye. The image is generated as individual shades of gray known-How the system works, researchers can decide MRI can effectively identify structural and cellular changes Death has been observed in the brains of Alzheimer’s patients. ships- In Alzheimer's disease, the hippocampus is often seen, even before the onset of clinical symptoms. Fifty-six participants had varying degrees of cognitive impairment. Measure and determine hippocampal size using MRI. The importance of an indicator of AD neuropathology. The results demonstrate that this analysis can be used for determination. Older people without dementia and people with Alzheimer's disease And people who have never experienced memory loss before go through determine the risk of these patients developing Alzheimer's disease Doctors can relieve symptoms long before symptoms appear ability to administer treatments that slow disease progression Disease a recent 2009 Ministry of Education survey Doctor of Radiology and Neurology, University of Pennsylvania is investigating the use of sodium MRI Alzheimer's disease detection This technique uses the same principle as the previous technique. but, use this technique instead of measuring hydrogen atoms Sodium is naturally abundant and is 23Na. To do this, select this ion Sodium is involved in the brain's ability to recognize and track tumors cell death. Participants were 5 healthy elderly people. 5 Adults and Suspected Alzheimer’s Disease. When nerve cells die, the spaces within the cells are destroyed. Therefore, its concentration increases the presence of sodium in the extracellular space increases signal intensity [Mellon et al., 2009]. Magnetic resonance imaging of Alzheimer's disease patients. The technology isn't perfect yet, but research is still ongoing. Do this and see if the signal strength increases. Fifty-six participants with varying degrees of cognitive impairment due to changes in ion concentration or quantity. Adjust and determine the strength of the signal increase due to changes in ion concentration or size [Chou, 2014].

**HEPATOTOXICITY**

**INTRODUCTION**

The liver performs an amazing variety of essential roles in the body's upkeep, operation, and homeostasis regulation. It is involved in practically all metabolic pathways that lead to growth, the prevention of disease, the supply of nutrients, the creation of energy, and reproduction [Sharma et al., 1991]. The metabolism of carbohydrates, proteins, and fats, detoxification, bile secretion, and vitamin storage are the liver's main tasks. Therefore, it is essential to maintain a healthy liver for one's general health and wellbeing [Subramaniam and Pushpangadan, 1999]. Damage to the liver brought on by chemicals is known as hepatotoxicity. Certain medications have the potential to harm the organ when taken in excess or occasionally even when administered within therapeutic parameters. Other chemical substances, including those employed in businesses and labs, natural substances (such microcystins), and herbal medicines can also induce hepatotoxicity. The term "hepatotoxins" refers to substances that harm the liver. The term "hepatotoxins" refers to toxins that harm the liver. The most frequent reason for a drug's removal from the market is liver damage, which has been linked to more than 900 different medications. Subclinical liver damage brought on by chemicals frequently only emerges as abnormal liver enzyme tests. 50% of all acute liver failures and 5% of all hospital admissions are due to drug-induced liver damage. Idiosyncratic medication responses frequently lead to liver transplantation or death in more than 75% of instances.

**HEPATOTOXIC DRUGS**

**Anti-Tubercular Drugs**

Rifampicin, isoniazid, and pyrazinamide, the first-line anti-tubercular medications, have the potential to be hepatotoxic. The liver breaks down these medicines. Ethambutol and streptomycin have not been linked to any liver damage reports. By using numerous drug regimens, adverse effects of antitubercular therapy might occasionally be amplified. Because of this, even while INH, Rifampicin, and Pyrazinamide alone have the potential to be hepatotoxic when administered alone, their toxic effect is increased when given together. The incidence of anti-TB associated hepatotoxicity is reported to range from 2% to 28% depending on the population under research and the hepatotoxicity diagnosis criteria [Girling, 1978].

**Rifampicin**

Hepatitis is more prevalent in patients receiving concomitant Rifampicin medication. This has been theorized as the result of increased formation of the hazardous metabolites from acetyl hydrazine (AcHz) caused by Rifampicin-induced cytochrome P450 enzyme-induction. Additionally, rifampicin speeds up the conversion of INH into the hepatotoxic compounds’ hydrazine and isonicotinic acid. Rifampicin reduces the plasma half-life of AcHz (an INH metabolite) and accelerates the oxidative clearance rate of AcHz, which is associated to the increased risk of liver necrosis brought on by the combined effects of INH and Rifampicin. Rifampicin also interacts with antiretroviral medications, affecting both the risk of hepatotoxicity and plasma levels of these medications [Padma et al., 1998; Tostmann et al., 2008].

**Isoniazid**

Isoniazid hepatotoxicity is a frequent side effect of antituberculosis treatment, and its severity can range from an asymptomatic increase in serum transaminases to hepatic failure necessitating liver transplantation. This does not result from elevated plasma isoniazid levels; rather, it seems to be an atypical reaction. Hepatotoxicity results from the cytochrome P450 enzyme's subsequent metabolization of INH to monoacetyl hydrazine, which is then converted into a hazardous substance. According to studies on human genetics, cytochrome P4502E1 (CYP2E1) plays a role in the liver toxicity of anti-tubercular drugs [Huang et al., 2003]. The CYP2E1 c1/c1 genotype is connected to an elevated amount of CYP2E1 activity, which could increase the synthesis of hepatotoxins. Isoniazid and Hydrazine stimulate CYP2E1 activity, according to research done on rats [Jenner and Timbrell, 1994; Jenner and Timbrell, 1995]. The activity of CYP1A2, 2A6, 2C19, and 3A4 is inhibited by isoniazid. The detoxification of hydrazine may include CYP1A2. By activating or inhibiting these enzymes, isoniazid can generate its own toxicity [Wen et al., 2002; Desta et al., 2001].

**Pyrazinamide**

By being converted to pyrazinoic acid by xanthine oxidase, pyrazinamide (PZA; pyrazoic acid amide) becomes 5-hydroxypyrazinoic acid. Pyrazinamide's serum half-life is not correlated with the duration of therapy, suggesting that it does not activate the enzymes necessary for its metabolism. Pyrazinamide-induced toxicity's mechanism, the enzymes involved, and whether the toxicity is brought on by the compound itself or its metabolites are all unknown. However, a study in human liver microsomes revealed that pyrazinamide has no inhibitory impact on the CYP450 isoenzymes [Maffei and Carini, 1980; Nishimura et al., 2004]. In a rat investigation, pyrazinamide decreased the activity of many CYP450 isoenzymes (2B, 2C, 2E1, and 3A).

**Non-Steroidal Anti-Inflammatory Drugs**

Non-steroidal anti-inflammatory drugs (NSAIDs), such as acetaminophen, Nimesulide, Diclofenac, and Ibuprofen are the mainstay of pharmaceutical treatment for the majority of rheumatological disorders. Both on prescription and off-label, they are widely used as analgesics and antipyretics. It is the primary factor in the hazardous drug-induced harm to multiple organ systems, including the well-known renal and gastrointestinal injuries. Centrilobular hepatic necrosis is caused by an overdose of the analgesic/antipyretic acetaminophen [Walker, 1997]. Clinically visible liver damage is rare (1–8 occurrences per 100,000 patient years of NSAID usage), but when it does happen, it can be devastating and be difficult to diagnose [Sgro et al., 2002]. Ibuprofen use, however, sharply increased between 1998 and 2000. The mechanism is believed to be immunological idiosyncrasy, and nearly all NSAIDs have been linked to liver damage. These injuries tend to be hepatocellular in origin [Zimmerman, 1990]. Due to related hepatotoxicity, a number of NSAIDs have been taken out of clinical practice [Connor et al., 2003]. According to Benichou (1990), the newer, more selective COX-2 inhibitors, such as celecoxib, rofecoxib, and nimesulide, are also linked to hepatotoxicity. However, celecoxib is thought to be less likely to cause hepatotoxicity. Examples of NSAIDs of different types that have been discontinued or withdrawn due to hepatotoxicity [Lewis, 2003].

**Mechanism of toxicity of NSAID’s**

Numerous in vitro animal models have recently been employed to research potential NSAID-related hepatotoxic pathways. Diphenylamine, which is frequently found in the structure of NSAIDs, uncouples oxidative phosphorylation, lowers hepatic ATP level, and causes hepatocyte damage, according to studies employing rat liver mitochondria and freshly isolated rat hepatocytes. Mitochondrial oedema resulted by diphenylamine, mefenamic acid, or diclofenac incubation. As another sign of the uncoupling of oxidative phosphorylation, the safranine-binding spectra to mitochondria shifted in spectral direction, indicating the loss of mitochondrial membrane potentials. Oligomycin, which inhibits ATPase, was added to provide protection from cellular harm. No appreciable oxidative stress (reduction in glutathione and lipid peroxidation) or rise in intracellular calcium concentration was observed in diclofenac-induced damage in hepatocytes. Administration of paracetamol results in the necrosis of centrilobular hepatocytes, which is characterized by nuclear pyknosis and eosinophilic cytoplasm and is followed by a significant amount of hepatic lesion. According to Masubuchi et al., [2000] and Bort et al., [1999], the covalent binding of N-acetyl-P-benzoquinoneimine, an oxidative byproduct of paracetamol, to sulphydryl groups of protein results in lipid peroxidative degradation of glutathione level and consequently causes cell necrosis in the liver.

**Diclofenac**

According to Mitchell et al., [1973], diclofenac hepatotoxicity is an archetype of idiosyncratic drug-induced liver injury (DILI). A threefold increase in transaminase levels has been documented in 5% of patients who take diclofenac on a regular basis, and approximately 15% of individuals experience high liver enzyme levels. Diclofenac is linked to liver damage that is primarily hepatocellular, but it has also been reported to cause cholestatic liver damage and cases that resemble autoimmune hepatitis [Aithal, 2004]. Diclofenac undergoes glucuronidation by UDP-glucuronosyltransferase-2B7 in addition to 4′-hydroxylation by cytochrome P450 2C9 to create an unstable acyl glucuronide. The latter is subjected to further oxidation by CYP2C8. Additionally, the production of 5-hydroxydiclofenac is catalyzed by CYP2C8. Both the benzoquinone imines generated from 5-hydroxydiclofenac and the diclofenac acyl glucuronide change proteins in a covalent manner; as a result, both decreased and enhanced CYP2C8 activity may potentially raise the risk of hepatotoxicity. Diclofenac acyl glucuronide is transported to biliary canaliculi by MRP2, and the metabolite builds up when MRP2 expression is reduced. Reactive metabolite buildup causes mitochondrial permeability transition and oxidative stress, which damage cells. Neoantigens are also produced as a result of reactive metabolites' covalent attachment to'self' proteins. A proinflammatory cellular milieu and a susceptible person could cause mild liver damage to worsen into DILI, or diclofenac adducts generated by dying hepatocytes could be phagocytosed by APCs and presented with MHC II molecules. Helper T cells activate and send out effector cells in response to neoantigen recognition. It is possible for hepatocytes to have diclofenac adducts and express MHC I molecules on their surface, which could cause cytotoxic T-cell-mediated liver damage. As an alternative, B cells may detect diclofenac adducts on the plasma membrane of hepatocytes, causing plasmacytes to develop into mature hepatocytes, antibodies to be secreted, and hepatocytes to be immunologically destroyed. APC stands for antigen-presenting cell; DILI stands for drug-induced liver injury; IL stands for interleukin; MRP2 stands for multidrug resistance protein 2; and TCR stands for T-cell receptor [Aithal, 2011].

**Sulindac**

The medication that has been most frequently linked to hepatotoxicity is sulindac. 91 cases of liver injury have been reported in published studies, with 43% of those cases exhibiting a cholestatic pattern (liver injury impairs bile flow, thus cases are typically characterized by itching and jaundice), 25% by a hepatocellular pattern, and the other cases by a mixed pattern of injury [Tarazi et al., 1993]. Four patients passed away, and 67% of the patients had jaundice. According to Bolder et al., (1999), sulindac competitively reduces canalicular bile salt transport, and this inhibition may be a factor in cholestatic liver damage.

**CURRENTLY USED BIOMARKERS**

**Diagnostic Biomarkers**

There are currently few biomarkers for DILI that can be used for diagnosis, monitoring, or early detection. The serum levels of alanine aminotransferase (ALT), aspartate aminotransferase, alkaline phosphatase (ALP), and gamma-glutamyl transferase have historically been used in clinical practice to detect liver injury. These markers also measure changes in tissue and cell integrity [Aithal et al., 2011]. The level of serum bilirubin determines how useful it is as a diagnostic marker. It may be difficult to distinguish between increased unconjugated bilirubin as seen with Gilbert illness and total bilirubin that is less than 40 mol/L without value for conjugated bilirubin. However, it is a diagnostic indicator of liver damage when its value is substantially beyond this threshold or when the increase is accompanied by an increase in conjugated bilirubin. The usage of bilirubin is more of a severity criterion, though (see the Severity Biomarkers section). The following case definitions for DILI were suggested by the EASL DILI recommendations, each of which includes one of the following thresholds:

A) An increase in serum ALT levels that is five times above the upper limit of normal (ULN) value.

B) An increase in serum ALP levels that is two times above the ULN value, particularly when there are concurrent increases in gamma-glutamyl transferase and no known bone pathology is to blame.or

c) The concomitant rise of total bilirubin concentration over 2 ULN and ALT elevation above 3 ULN (2)

These conventional biomarkers have a number of shortcomings that prevent them from being the ideal biomarkers in actual practice, despite the fact that they can represent hepatic lesions and be helpful for the diagnosis of severe DILI. Aspartate aminotransferase and ALT levels in the serum are frequently utilized as biomarkers of hepatic injury, despite the fact that their rise can also be indicative of cardiac and muscle damage, respectively. These indicators also make it impossible to distinguish DILI from other liver injury etiologies or pinpoint its precise cause. Additionally, there is little relationship between liver enzyme levels and lesion severity and histological patterns [Devarbhavi, 2012]. The diagnosis of DILI is currently made primarily using chronological criteria, clinical criteria, and the exclusion of other competing causes.

It is frequently an elimination diagnosis when there is a lack of specificity [Fontana et al., 2009; Larrey et al., 2017]. Methods based on ratings given to the pertinent factors can be used to determine causality. Based on ratings, a number of causality assessment techniques (CAM) have been created. The Roussel Uclaf Causality Assessment Method (RUCAM) is the primary one. There are several methods for determining causality, such as the American Drug-Induced Liver Injury Network system, which is based on a calculation of causality's probability rather than on scores [Fontana et al., 2009]. The most widely utilized CAM is the freshly improved RUCAM.

**Determination of the Drug**

Paracetamol serves as a prototype because of its straightforward, predictable, and dose-dependent toxicity mechanism. Plasma paracetamol levels are directly connected with liver toxicity (>200 g/L 4 h or >100 g/L 8 h after intake).

**Specific Autoantibodies**

Certain antibodies are linked to the hepatotoxicity of several medications. They make excellent diagnostic markers since they have excellent specificity and sensitivity together. As demonstrated by anti-cytochrome 1A2 with dihydralazine, anti-cytochrome 3A with anti-epileptics, and anti-cytochrome 2E1 with halothane [Larrey et al., 2017], this is also true for anti-mitochondrial antibodies type 6 with isoniazid. Anti-epoxide hydrolase antibodies, a particular marker for the liver toxicity of germander (Teucrium chamaedrys), are a further intriguing example. An aid to weight loss was granted a commercial license for this medicinal plant in 1986 after it was first used as an antipyretic and analgesic for abdominal pain [Larrey and Faure, 2011; Teschke et al., 2016]. In a short period of time, the pharmacovigilance center gathered more than 30 cases of drug-related liver impairment, including fulminant hepatitis. As a result, it has been taken off the market and is no longer permitted for free sale. Then, the mechanism of hepatotoxicity was established: CYP 3A oxidizes Germander to produce reactive metabolites, which are the target of anti-epoxide hydrolase antibodies in the blood [Larrey and Faure, 2011; Teschke et al., 2016].

**Detection of Reactive Adducts in Serum**

The production of harmful reactive metabolites from the drug is another illustration of a biomarker based on one of the primary toxicity mechanisms [Larrey et al., 2017].

This harmful metabolite has the ability to stably bind to proteins as well as other organelles and molecules. A blood adduct that is formed by the reactive protein-reactive metabolite complex can be identified. It has been proven that patients with paracetamol-induced liver injury can have a paracetamol metabolite-protein adduct found in their blood, although this method of diagnosis is not currently used in clinical settings and is therefore not usually required [James et al., 2009]. Another recent instance is the discovery of a hazardous metabolite made from pyrrolizidine alkaloids in the blood or certain urine samples [Larrey and Faure, 2011]. The traditional Chinese medicine "Tusanqi," which is used as a painkiller and is often risk-free but has resulted in a string of 50 cases of sinusoidal obstruction syndrome, is the source of the development of this biomarker [Teschke et al., 2016]. The production of this composition accidentally substituted two plants, confusing the safe Sedum aizoon with the deadly Gynura segetum, which contains alkaloids, leading to toxicity [Teschke et al., 2016]. With a beneficial evolution, a biomarker of pyrrolizidine alkaloids was presented, originally tested in rats, and then in a patient with sinusoidal obstruction syndrome. This allowed the diagnosis to be made with confidence and had a specificity of 95.8% and a sensitivity of 100%.

During the first 40 days, the amount of adducts of reactive pyrrole-protein reactive metabolites declines quickly, but they are still detectable in the blood for about 300 days [Larrey and Faure, 2011; Lin et al., 2011; Teschke et al., 2016].

**NEUTRALISED CANDIDATE BIOMARKERS**

**The miRNA 122**

Short RNAs called microRNAs (miRNAs) influence the translation of proteins and the expression of genes, and they play a critical role in the control of cellular activities. Hepato-specific miR-122 and miR-192 are circulating microRNAs. A possible early indicator of liver damage caused by viruses, alcohol, or toxic substances is microRNA 122. MicroRNA 122 is more vulnerable to paracetamol intoxications and increases before transaminases do.In contrast to ALT, microRNA 122 levels in the blood do not rise in cases of muscle injury. Consequently, it is more precise in this instance. MiRNAs, like miRNA-122, have the potential to offer both improved sensitivity and specificity in predicting, monitoring, and classifying DILI, according to Howell's recent review article [Howell et al., 2018].

**Cytokeratin 18**

The liver contains a lot of this non-specific cytoskeleton protein. In the event of hepatocyte necrosis, it is released. It rises sooner than ALT does in cases of liver toxicity. It may also serve as a predictive indicator of liver damage because it is elevated more in paracetamol overdose patients who die or get liver transplantation compared to those who recover on their own [Thulin et al., 2014; Church and Watkins, 2017; Kullak-Ublick et al., 2017; Church et al., 2019]

**CANCER**

Cancer is an intricate illness that causes a variety of temporal and spatial changes in cell physiology that ultimately result in malignant tumors. The biological hallmark of the illness is abnormal cell growth, or neoplasia. For the majority of cancer patients, the primary cause of morbidity and mortality is tumor cell invasion of nearby tissues and distant organs. The biological mechanism by which healthy cells become cancerous has been the focus of extensive research in the biomedical sciences for many years.

Cancerous tumors spread into, or invade, nearby tissues and can travel to distant places in the body to form new tumors (a process called [metastasis](https://www.cancer.gov/Common/PopUps/popDefinition.aspx?id=CDR0000046710&version=Patient&language=en)). Cancerous tumours may also be called [malignant](https://www.cancer.gov/Common/PopUps/popDefinition.aspx?id=CDR0000045772&version=Patient&language=en) tumors. Many cancers form solid tumors, but cancers of the blood, such as [leukemias](https://www.cancer.gov/Common/PopUps/popDefinition.aspx?id=CDR0000045343&version=Patient&language=en), generally do not. Benign tumors do not spread into, or invade, nearby tissues. Benign tumors typically don't come back after removal, whereas cancerous tumors can. However, benign tumors can occasionally grow to be quite large. Some, like benign brain tumors, can have grave side effects or even be fatal. Metastatic cancer is a type of cancer that has spread from the site of its initial formation to another location in the body. Metastasis is the process by which cancer cells spread to different areas of the body. The primary or original cancer's name and cancer cell type also apply to metastatic cancer. For instance, breast cancer that spreads to the lung and develops a tumor is considered metastatic breast cancer rather than lung cancer. Metastatic cancer cells typically resemble the original cancer's cells when viewed under a microscope. Additionally, there are some molecular similarities between metastatic cancer cells and the original cancer cells, such as the presence of particular chromosome changes.

When the DNA (deoxyribonucleic acid), or cellular blueprints, of normal cells are damaged, cancerous cells develop. Every cell has DNA, which controls all of the activities of the cell, including growth, death, protein synthesis, etc. In a typical cell, when DNA is harmed, the cell either repairs the damage or dies. Cancer cells do not die because the damaged DNA is not repaired. Instead, it produces more of these dysplastic cells with DNA. All of these new cells share the original cancer cell's incomplete DNA.



**A series of genetic changes that lead to cancer**

In some cases, treatment may help prolong the lives of people with metastatic cancer. In other cases, the primary goal of treatment for metastatic cancer is to control the growth of the cancer or to relieve symptoms it is causing. Metastatic tumors can cause severe damage to how the body functions, and most people who die of cancer die of metastatic disease.  Biomarker testing is a way to look for genes, proteins, and other substances (called [biomarkers](https://www.cancer.gov/Common/PopUps/popDefinition.aspx?id=CDR0000045618&version=Patient&language=en) or tumor markers) that can provide information about cancer. Each person’s cancer has a unique pattern of biomarkers. Some biomarkers affect how certain cancer treatments work. Biomarker testing may help you and your doctor choose a cancer treatment for you. Biomarker testing is a way to look for genes, proteins, and other substances (called [biomarkers](https://www.cancer.gov/Common/PopUps/popDefinition.aspx?id=CDR0000045618&version=Patient&language=en) or tumor markers) that can provide information about cancer. Each person’s cancer has a unique pattern of biomarkers. Some biomarkers affect how certain cancer treatments work. Biomarker testing may help you and your doctor choose a cancer treatment for you.

There are also other kinds of biomarkers that can help doctors diagnose and monitor cancer during and after treatment.

**The symbol and Idyllic Biomarker of cancer**

The eight biological characteristics that define cancer serve as an organizing principle for making sense of the complexity of neoplastic disease. These characteristics were acquired during the multi-step development of human tumors.

1. Sustaining proliferative signaling

2. Evading growth suppressors

3. Resisting cell death

4. Enabling replicative immortality

5. Inducing angiogenesis

6. Activating invasion and metastasis

7. Reprogramming energy metabolism (emerging)

8. Escaping immune destruction (emerging)

Genome instability, which produces the genetic diversity that speeds up their acquisition, and inflammation, which promotes multiple hallmark functions, underlie these hallmarks. In addition to cancer cells, tumors also display another level of complexity: they contain a variety of recruited, seemingly normal cells that aid in the development of distinguishing characteristics by establishing the "tumor microenvironment."

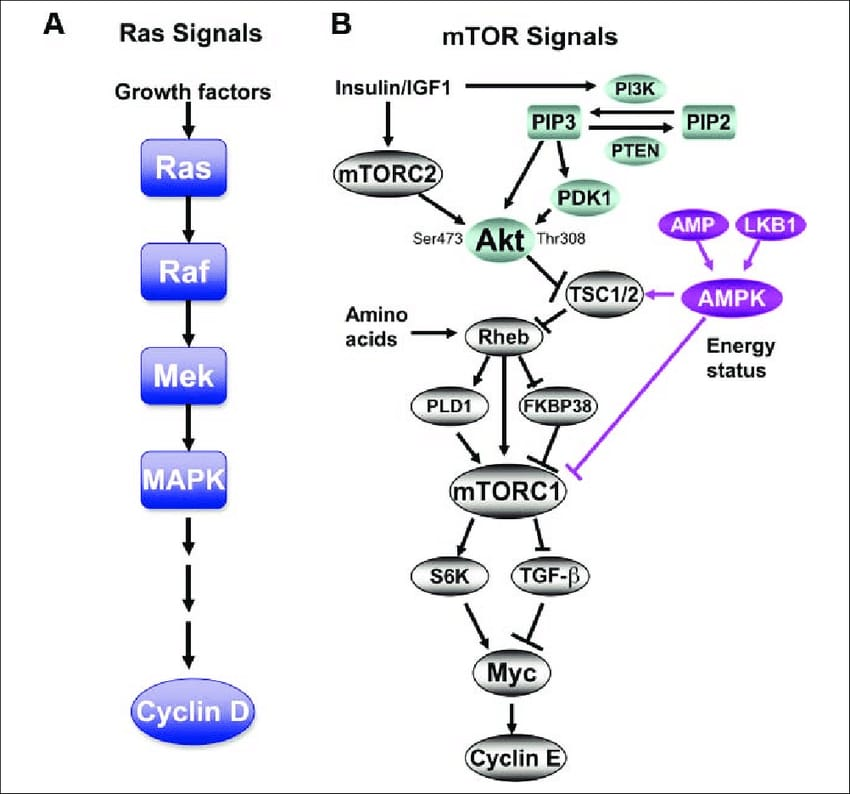
These characteristics should be taken into account when looking for biomarkers to help with cancer diagnosis and treatment.

**Diffusive Cancer Biomarkers**

Biomarkers can be found on fluid biopsies of body fluids, mostly circulating plasma, as an alternative to cancer tissue biopsy. This section will cover exosomes or microvesicles, circulating tumor cells, and circulating nucleic acids as three major categories of circulating biomarkers.

Exposed tumor tissue can be examined for biomarkers, particularly for prognostic and theranostic purposes. In order to identify patients with particular types of epidermal growth factor receptor (EGFR) gene mutations in non-small cell lung cancer (NSCLC), EC- Endometrial cancer, Cervical cancer, Colorectal which will inform treatment choices, the FDA has approved the first liquid biopsy companion diagnostic that also uses next-generation sequencing (NGS) technology.

Biomarkers that can be detected in blood or other bodily fluids, such as urine, saliva, cyst fluid, ascites, and pleural fluid, may be very helpful in this situation. Active secretion can cause cancer biomarkers to become visible in the blood circulation or cellular leakage in the tumor microenvironment from cancer cells or supporting tissue. In addition to serving as a gauge of tumor load and metastatic potential, circulating biomarkers such as proteins and auto-antibodies, nucleic acids (cell-free DNA and RNA), circulating tumor cells, and microvesicles dispersed in the blood stream can also be used to investigate specific molecular alterations in a tumor. The current emphasis on so-called "liquid biopsies" is due to the fact that these biomarkers offer a potent substitute to invasive biopsies of particular organs for molecular analysis [Marrugo-Ramírez., 2018].



**MMP**

The breakdown of extracellular matrix is facilitated by a family of zinc-dependent endopeptidases known as MMPs (matrix metalloproteinases). Six subclasses of MMPs exist: membrane-type MMPs, stromelysins, matrilysins, collagenases, and gelatinases. Both physiological and pathological processes, such as the invasion and spread of malignant cancers, depend on them. Additionally, according to Kim et al., (2007), they exist in both ectopic and eutopic endometrium. The basal membrane is specifically important for the degradation by MMP-2 (gelatinase A), which is present. MMP expression has started to become overexpressed in endometrium and numerous malignant tumors [Chung et al.,2002].

MMP-9 (gelatinase B) also progresses type IV collagen and is present in many different cancer types, including malignant cancer and the stroma around it. Similar to gelatinase A, gelatinase B is present in both hyperplastic endometrium and endometrial carcinoma in addition to typical endometrium in the proliferative phase [Graesslin et al., 2006]. As a result, MMP-2 and MMP-9, among other MMPs, demonstrated to be particularly significant factors in the development and invasion of endometrial cancer [Amalinei et al.,2011].

**Table 5. Functions of MMP**

|  |  |  |
| --- | --- | --- |
| **Physiological function** | **Pathological function** | |
| Angiogenesis | Arthritis | Various Sclerosis |
| Apoptosis | Alzheimers disease | Nephritis |
| Blastocyst implantation | Atherosclerosis | Neurological disease |
| Bone remodeling | Break down of blood-brain barrier | Osteoarthritis |
| Cervical dilation | Cancer | Periodontal disease |
| Embryonic development | Cardiovascular disease | Rheumatoid |
| Endometrial cycling | Central nervous system disorder | Skin ulceration |
| Hair follicle cycling | Corneal ulceration | Sorbys fundus disease |

**VEGF**

Vasculogenesis and angiogenesis are mediated by vascular endothelial growth factors. The development of new blood vessels, the spread of solid tumors, and metastatic growth are all well-established processes [Bergers and Benjamin, 2003]. Two vascular endothelium-expressed high-affinity transmembrane tyrosine kinase receptors, VEGFR-1 and VEGFR-2, inhibit VEGF [Talvensaari et al., 2005]. Numerous cancers, including colorectal, head and neck, ovarian, and endometrial cancer, have tumors that grow and metastasize more quickly when VEGF is overexpressed in the tumor cells [Sivridis et al., 2002].

**Oncogene and tumour suppressor genes**

**PTEN**

Phospho Along with the tensin cytoskeletal proteins, tensin homologue genes have formations of protein tyrosine phosphatases. Other proteins' serine, threonine, and tyrosine residues are dephosphorylated in vitro by PTEN. The cephalic and caudal regions exhibit increased cell proliferation and overgrowth due to the PTEN gene. PTEN is primarily active in the female reproductive tract when proliferative endometrial lesions have developed [Hongbo et al.,2001]. Human cancers like melanoma, glioma, prostate, and endometrial carcinoma are used to divert the PTEN gene through deletion or mutation. According to George et al., (2000), PTEN inactivation occurs at the beginning stages of endometrial carcinogenesis and leads to the development of cytologic atypia in hyperplasia. Its primary effects include inhibition of growth factor-stimulated MAPK signaling pathway, up-regulation of cyclin dependent kinase, pro-apoptotic activity involving caspases, down-regulation of anti-apoptotic protein Bcl-2, and up-regulation of PI3K signaling pathway [Wu et al., 2003].

**PI3K**

In cellular growth, transformation, adhesion, survival, motility, and Ras-mediated cell proliferation, the phosphatidylinositol 3-kinase (PI3K) pathway is crucial. In several tumor lineages, including EC, aberrations at multiple nodes frequently initiate the PI3K signals downstream from receptor tyrosine kinases (RTK) [Hennessy et al., 2005]. When PTEN activates PI3K, phosphatidylinositol 3,4,5-triphosphate (PIP3) is produced from phosphatidylinositol 4,5-bisphosphate (PIP2) as a second messenger. Ras/mitogen-activated protein kinase (MAPK) and PI3K pathways can interact in both directions, suggesting that the two pathways may work together to determine functional outcomes [Gupta et al., 2007].

**KRAS**

A Potential Therapeutic Target for the Treatment of Cancer is KRAS. The most frequently mutated oncogene in human cancer is known as Kirsten rat sarcoma 2 viral oncogene homolog (KRAS). KRAS is a high-priority therapeutic target because the progression of many malignancies depends on its continued expression and signaling.

AMG510 (sotorasib), an allele-specific covalent inhibitor of KRAS (G12C) with notable clinical responses in a variety of tumor types, has been approved by the Food and Drug Administration (FDA) [Janes et al., 2018]. Additionally, a novel targeted therapy is offered by MRTX1133, a selective non-covalent inhibitor of KRAS (G12D) [Vasan et al., 2014]. The next challenge in this new era of KRAS mutant targeting will be to comprehend and circumvent medication resistance processes. In this review, we outline the most recent therapeutic methods for KRAS mutant malignancies and talk about the mechanisms of KRAS mutant therapy resistance as well as potential solutions. malignancies caused by the KRAS mutation KRAS mutations are prevalent in a wide range of cancers, including 45% of CRC cases in the US and 49% of CRC cases in China, 90% of pancreatic ductal adenocarcinomas (PDAC) in the US and 89% of LUADs (a subtype of non-small-cell lung cancer) in the US and 13% of LUADs in China [Hofmann et al., 2022]. KRAS is made up of two isomers, KRAS4A and KRAS4B, which are produced by the KRAS gene's selective splicing. The mutations KRAS (G12D), KRAS (G12V), KRAS (G12C), KRAS (G13D), KRAS (G12R), and KRAS (G12A) or KRAS wild-type amplification are the most common subtypes of mutant KRAS. The stability of the arginine residue hydrolysis transition state is destroyed by genetic modifications to G12 or G13 [Xue et al., 2020]. KRAS (G12C) mutations are present in 41% of LUAD, while the two most prevalent alleles in CRC and PDAC are KRAS (G12D) and KRAS (G12V). The prevalence of KRAS mutations differs among different types of human malignancies. Notably, PDAC has less copies of other KRAS alleles such G12R. Although KRAS mutations are the cause of the tumor type, the codons and frequency of mutations differ depending on the tissue type.

**KRAS biology: functions and signalling pathways**

By taking part in central carbon metabolism, enhancing glucose uptake and glycolysis to enhance the nutrients flux and stimulating various branching biosynthetic pathways, KRAS signaling gives cancer cells a competitive advantage. By stimulating phagocytosis, it also controls the overall mitochondrial content and function, and the damaged mitochondria slow the growth of tumors. The synthesis of nicotinamide adenine dinucleotide phosphate (NADPH) increases as a result of KRAS's promotion of alternative glutamine catabolism [Uprety, 2020]. When cells are cultivated in buffered brine deficient in key nutrients, the limited supply of mitochondrial substrates can result in dangerously high levels of reactive oxygen species (ROS) and depleted nucleotide pools. KRAS regulates pinocytosis to deal with this situation. To sustain TCA cycling and nucleotide synthesis, autophagy flux supplies glutamine and glutamate to KRAS-driven cancer cells [Dai et al., 2020].

**MAPK**

The RAS-RAF-MEK-ERK signaling pathway is also a word for the mitogen-activated protein kinase (MAPK) pathway. Activated by upstream growth factors and their receptors, such as tyrosine kinases, G protein-coupled receptors, cytokine receptors, and integrins, it controls cell division and proliferation. Endometrial cancer was the source of the activating KRas mutations that first caused oncogenic changes in the Ras/MAPK pathway [Urick et al., 2011]. The increased level of growth factors and their receptors in cancer frequently leads to an autonomous and constitutive activation of MAPK signaling [Cheung et al.,2011].

**TGF-β**

Emerging factor that converts Superfamily cytokines regulate a wide range of biological processes, including cell division, migration, survival, and apoptosis [Derynck, 2003]. TGF- works by connecting to a heteromeric complex of type I (RI) and type II (RII) transmembrane serine/threonine kinase. The TGF-type I receptor then binds ligand to the RI/RII complex, phosphorylating Smad2 and Smad3, the so-called receptor-regulated Smads (R-Smads). Once Smad4 and phosphorylated R-Smads form a heteromeric complex, Smad4 translocates into the nucleus to control the transcription of TGF-responsive genes. In addition to MAPK, PI3K, and RhoA pathways, there are numerous Smad-independent pathways. The TGF-signaling pathway is crucial for female reproduction, and its dysregulation may be the cause of grave consequences, particularly cancers and diseases that affect the reproductive system [Edson, 2010]. TGF- expression in EC was disrupted at both the mRNA and protein levels, which may have an impact on tumor development and cell survival.



**Major function of TGF-β superfamily signaling in female reproduction Cell proliferation**

**PCNA**

In cells that are in the DNA synthesis phase of the cell cycle, the proliferating cell nuclear antigen (PCNA), a marker of cell proliferation, is expressed in the nuclei. Proliferative behavior can be used to grade benign lesions and altered neoplasms [Marion et al., 2012]. PCNA performs essential cellular functions such as chromatin remodeling, DNA repair, sister-chromatid cohesion, and cell-cycle regulation in addition to DNA replication (Ivaylo and Thomas, 2009). Endometrial hyperplasias and carcinomas both showed increased expression of PCNA [Amalinei et al.,2011].

**Cell cycle gene**

**Cyclin D1**

Cyclins are essential elements in the control of the cell cycle from the G1 to the S phase. By activating their cdk partners, which phosphorylate and transition the cell cycle from G1 to S phase, D cyclin regulates the progression of the cell cycle with specificity. Cyclin D1 overexpression in endometrial cancer is caused by hyperesterogenesis and hyperplasia. One of the typical features of tumor growth is the deregulation of the gene cyclin D1, which is involved in the control of the cell cycle [Nishimura et al.,2004].

**Signalling pathways**

**Wnt/β-catenin**

The wnt signaling pathway is a collection of pathways that includes proteins engaged in signal transduction via cell surface receptor. During embryogenesis and tissue homeostasis, it is one of the main techniques for promoting cell proliferation, cell polarity, and cell fate determination [Logan and Nusse, 2004]. The main focus of Wnt pathway research is canonical Wnt signaling, which regulates the transcriptional co-activator -catenin, which regulates important developmental gene expression programs. The accumulation and translocation of -catenin to the nucleus, where it binds with T-cell factor/lymphoid enhancer factor, allows the activation of gene mutation in ovarian cancer [Shi and Massague, 2003]. The canonical Wnt pathway is organized by the multifunctional protein -catenin, which is also a crucial structural element of cadherin-based adhesion junctions [Valenta et al., 2012]. Numerous subcellular localizations of -catenin exist; at the cell membrane, it maintains cell-to-cell adhesion between E-cadherin, inside the cytoplasm at tightly regulated levels through phosphorylation through protein complexes, and in the free unphosphorylated form within the nucleus that binds to transcription factors inducing target genes transcription [Kim et al., 2013].

**E-cadherin**

E-cadherin is a transmembrane protein that controls cell-cell adhesion and is essential for the structure of epithelial tissue organization and maintenance. Reduced expression of E-cadherin, a gene that plays an invasive cancer suppressor role, is thought to be largely responsible for decreased cell-to-cell adhesion in epithelial cells. In endometrial and other carcinomas, loss of E-cadherin expression may be linked to decreased cell-to-cell adhesion and increased invasive and metastatic potential [Yalta et al., 2009].

**Epithelial cell adhesion molecule (EpCAM)**

A glycoprotein labelled EpCAM can be found on chromosome two. According to Trebak et al., (2001), the molecule is associated with signal transduction-dependent epithelial morphogenesis, homotypic intercellular adhesion of epithelial cells, and the potential metastasis of epithelial neoplasias. EpCAM is a diagnostic marker for many cancers because it has been linked to poor prognosis and overexpression in carcinomas [Calabrese et al., 2001]. EpCAM antibodies and broad-expression EpCAM have been used to treat patients with metastatic carcinomas [Seligson et al.,2004].

**Hormone receptors**

**Progesterone receptor (PR)**

(Steroid hormone) is crucial for coordinating the physiology of female reproduction in normal mammals. Through PR-dependent transcriptional activity, progesterone controls a number of signaling pathways. It is expressed in a number of human organs, including the ovary, uterus, and mammary gland. In EC, oncogenes and the Smad pathway act to suppress PR expression, which results in gene mutation [Lee et al.,2006].

**Estrogen receptor (ER-α)**

The sex steroid hormones progesterone and estrogen play a major role in regulating the female reproductive system's operation. The nuclear receptor superfamily includes estrogen receptors (ER), which are crucial in controlling a variety of reproductive processes [Mangelsdorf et al., 1995]. As soon as the ligand is activated, the ER forms a dimer and binds to particular DNA sequences called estrogen-responsive elements, which are found in the promoters of target genes [Kenneth et al., 2000]. Both the male and female reproductive tracts, breast, and brain express the two ER isoforms, ER- and ER-. In EC cells, ER- predominates and frequently influences genes that promote growth. The prostate epithelial cells exhibit the highest levels of ER- expression [David et al.,2011].

**Inflammatory marker**

**TNF-α**

A potent pro-inflammatory cytokine called tumor necrosis factor (TNF) is typically produced by activated macrophages and other cells in response to tissue damage or persistent inflammation [Diez et al., 1995]. TNF- has the potential to play a role in cancer and metastasis through its pro-inflammatory actions because it triggers ROS, which can damage DNA and prevent DNA repair [Balkwill, 2002]. TNF- has been linked to the cyclic shedding and regeneration of endometrium [Haider and Knofler, 2009], and patients with endometrial carcinoma have been found to have elevated TNF- serum concentrations [Chopra et al.,1997].

**COX-2**

The enzyme cyclooxygenase, which is stimulated by many factors including mitogens and tumor promoters, is the rate-limiting step in the conversion of arachidonic acid to prostaglandin synthesis. Numerous human epithelial cancer cells, as well as eutopic and ectopic endometrial tissue, have been found to have aberrant COX-2 expression, according to some earlier studies [Fagotti et al., 2004]. According to Ferrandina et al., (2002), COX-2 overexpression is an unfavorable outcome for cervical carcinoma patients and a key indicator of how well chemotherapy is working. Compared to the expression in the normal endometrium, COX-2 is highly expressed in endometrial hyperplasia and carcinoma [Comerci et al., 2000].

**Circulating Plasma/Serum-Nucleic acids**

Human blood contains Circulating free DNA in a variety of forms, including naked (unbound) DNA, DNA linked to histones (mononucleosomes and oligonucleosomes), DNA bound to plasma proteins, and DNA encapsulated in apoptotic bodies. Typically, circulating nucleosomes are quantified using ELISAs, or enzyme-linked immunosorbent assays. Elevated levels of circulating nucleosomes have been seen in the blood, regardless of the histological cancer type [Holdenrieder et al., 2008]. Since they cannot distinguish between benign and malignant breast lesions and uterus lesions, this increase in breast tumors does not appear to be cancer-specific [Roth et al., 2010]. On the basis of the correlation between the concentrations of circulating nucleosomes and the activities of serum caspases-3 and -7, which are involved in the apoptotic pathway, circulating nucleosomes can be used as a sensitive marker for apoptotic cell death. According to research conducted in my lab, the progression of cancer may be related to changes in the apoptosis-related deregulation of proteolytic activities and elevated serum nucleosome levels [Roth et al., 2010]. Circulating nucleosome levels dropped in cancer patients after mastectomy and rose in those who experienced recurrence. Significantly, their quantification may be a leukopenia predictor [Kuroi et al., 2001].

**Apoptosis**

Apoptosis (programmed cell death) has been linked to the regulation of several physiological processes, including immune system control, normal cell turnover, embryonic development, and hormone-dependent tissue atrophy.

**Extrinsic pathway**

The extrinsic pathway is activated by the ligation of transmembrane death receptors (CD95, TNF receptor, and TRAIL receptor) to cause membrane-proximal (activator) caspases (caspase-8 and 10), which in turn cause (effector) caspases like caspase-3 and 7 to cleave and become active. When a ligand binds to these receptors, the receptors oligomerize and FADD and/or TRADD, two adaptor proteins with death domains, are recruited. As a result, Pro-Caspase-8 and Pro-Caspase-10 are recruited by FADD during its death effector domain. Pro-Caspase-8 and Pro-Caspase-10 are cleaved as a result of the death-inducing signaling complex (DISC) being formed in the cytosol close to a death receptor [Zimmerman et al.,2002].

**Intrinsic pathway**

The intrinsic pathways involve rupturing the mitochondrial membrane and releasing mitochondrial proteins, such as Smac/DIABLO and cytochrome c, which cooperate with Apaf-1 to cause caspase-9 activation. The opposite behaviours of the pro- and anti-apoptotic Bcl-2 family members are coordinated with the permeabilization of the mitochondrial membrane. Bak and Bax are two examples of multi-domain proapoptotic Bcl-2 proteins that can interact with the BH3-only Bcl-2 protein Bid later.

**Caspases 3 and 9**

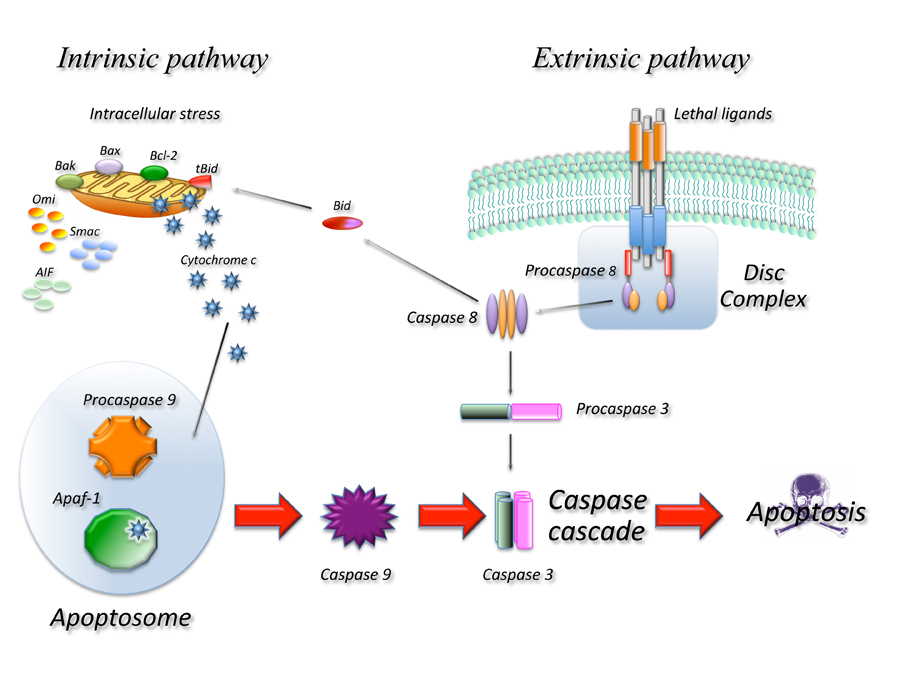
The large family of cysteine proteases known as caspases is primarily responsible for apoptosis's execution. In mammals, it is primarily divided into caspases-3, -6, -7, and 9, which are further divided into initiator (caspases-8 and -9) and executioner (caspases-3, -6, and -7). The inactive procaspase-3 form of caspase-3, a crucial apoptotic marker, is found in the cytosol of cells. It cleaves an inhibitor of caspase activated DNAse in order to enter the nucleus and fragment nuclear DNA. The functionally necessary starter of the apoptotic cascade is caspase-9. To cause apoptosis, caspase-9 initiates a caspase signaling cascade. Caspase 3 and 9 levels were found to be declining in EC [Zhao et al., 2006].

**Bax and Bcl-2 proteins**

The homodimerization of the pro-apoptotic protein Bax, which promotes cell death, is necessary. It induces apoptosis in response to genotoxic stress and safeguards cells from developing into cancerous tumors. Bax also forms heterodimers with Bcl-2 and inhibits Bcl-2's ability to prevent apoptosis. Few malignancies (colorectal, endometrial, and hemopoietic maligancies) that lack the expression of Bax led to the insertion or deletion of a single residue. This mutation could result in the loss of the immunodetctable Bax protein and a proximal frameshift [Noriaki et al., 2002].

**Bcl-2**

An oncogene termed Bcl-2 is found on chromosome 18. Bcl-2 is only found in the endoplasmic reticulum membrane and the outer mitochondrial nuclear envelop. Typically, endometrial epithelial cells express Bcl-2. The patterns of expression change throughout the menstrual cycle, indicating that hormones only slightly control expression. Since endometrial hyperplasia is characterized by increased Bcl-2 expression, it has been hypothesized that this increased expression may help endometrial carcinoma develop. Endometrial carcinogenesis may be associated with sustained Bcl-2 expression and decreased Bax expression that result in an increase in the Bcl-2/Bax ratio, which may inhibit apoptosis [Noriaki et al., 2002].



**Mitochondrial intrinsic and extrinsic apoptotic pathway**

**Circulating tumor cells**

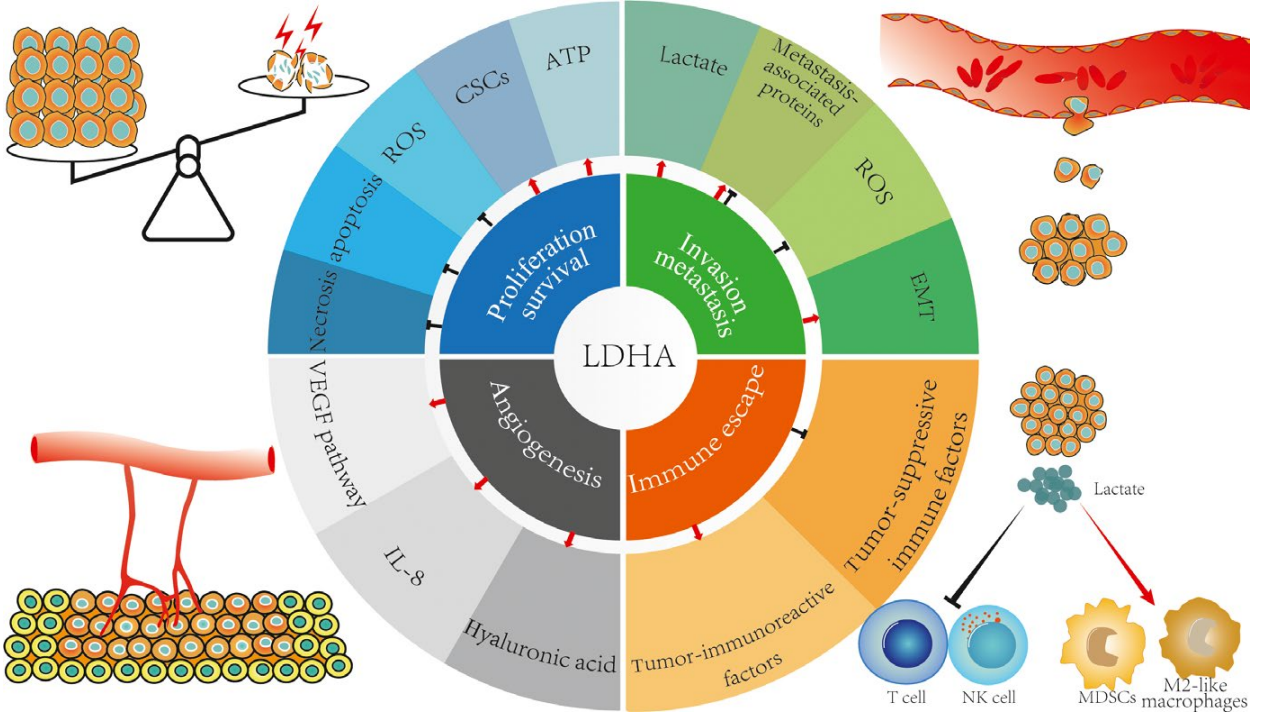
Circulating tumor cells (CTCs) are reliable indicators of the development of metastasis and potential prognostic biomarkers of cancer. Since the nineteenth century, it has been known that the development of distant metastasis requires the presence of CTCs. CTC counts have been employed in clinical trials as biomarkers for prognostic stratification and assessment of disease response to treatment. The quantity of CTCs may be helpful for early disease prognosis, for identifying patients who need adjuvant therapy, or for follow-up to look for relapses. The molecular and genetic characterization of CTCs is the foundation for additional clinical applications. In numerous lung cancer and breast cancer clinical trials, these applications have proven to be helpful.

**CA-125**

The second most fatal gynecological cancer is ovarian cancer. For the past four decades, the predominant ovarian cancer marker has been the tumor biomarker CA125. There has been extensive research done on ovarian cancer screening, diagnosis, and progression using the biomarker CA125. The MUC16 molecule's CA125 epitope serves a variety of physiological purposes. With 22,152 core amino acids and a molecular weight of about 2.5 MDa, MUC16 is a large glycoprotein. With a theoretical mass of around 20 MDa, MUC16 has a sizeable fraction that is O- and N-linked glycosylated. It has several different domains, including the transmembrane domain, a cytoplasmic tail of 32 amino acids rich in tyrosine, threonine, and serine residues used for potential phosphorylation, and 60 tandem repetitions of 156 amino acids at the amino-terminus [Hattrup et al., 2008]. The CA125 antigen can often be detected throughout the body. According to Hattrup., et al., (2008), CA125 is created and secreted by endocervical cells and is found in the cervical mucus of healthy women. The growing fetus's amniotic fluid and chorionic membrane contain large amounts of CA125 as well [O'Brien et. al., 1986]. In addition, it is found in human milk, airway epithelial cells, respiratory glands, and bronchial mucus [Hanisch et al., 1985; Matsuoka, 1990]. The OC125 monoclonal antibody was investigated for reactivity with various foetal and adult tissues. Coelomic epithelium, foetal amniotic, and adult tissues derived from coelomic and Mullerian epithelia all expressed CA125. So, in addition to these tissues, CA125 is also expressed in the endocervix, endometrium, pleura, pericardium, peritoneum, secretory mammary glands, apocrine sweat glands, intestines, lungs, and kidneys. Adenocarcinomas of the endocervix, endometrium, mesotheliomas, and fallopian tubes also reacted to OC125. In the embryonic development of the ovaries, CA125 is present; however, it disappears during development and is later re expressed in ovarian neoplasms. Elevations may be observed in the peritoneal and pleural epithelial and ascites fluids because CA125 is produced by tissues descended from coelomic epithelium [Buamah, 2000]. More significantly, ovarian cancer cells break and shed the extracellular component of this glycoprotein, which makes it detectable in bodily fluids such serum and peritoneal fluid [Szubert et al., 2012] and amniotic fluid [Seong, 2016]. Given the sensitivity of this CA-125 biomarker, ovarian cancer treatment can be determined.

**LDH**

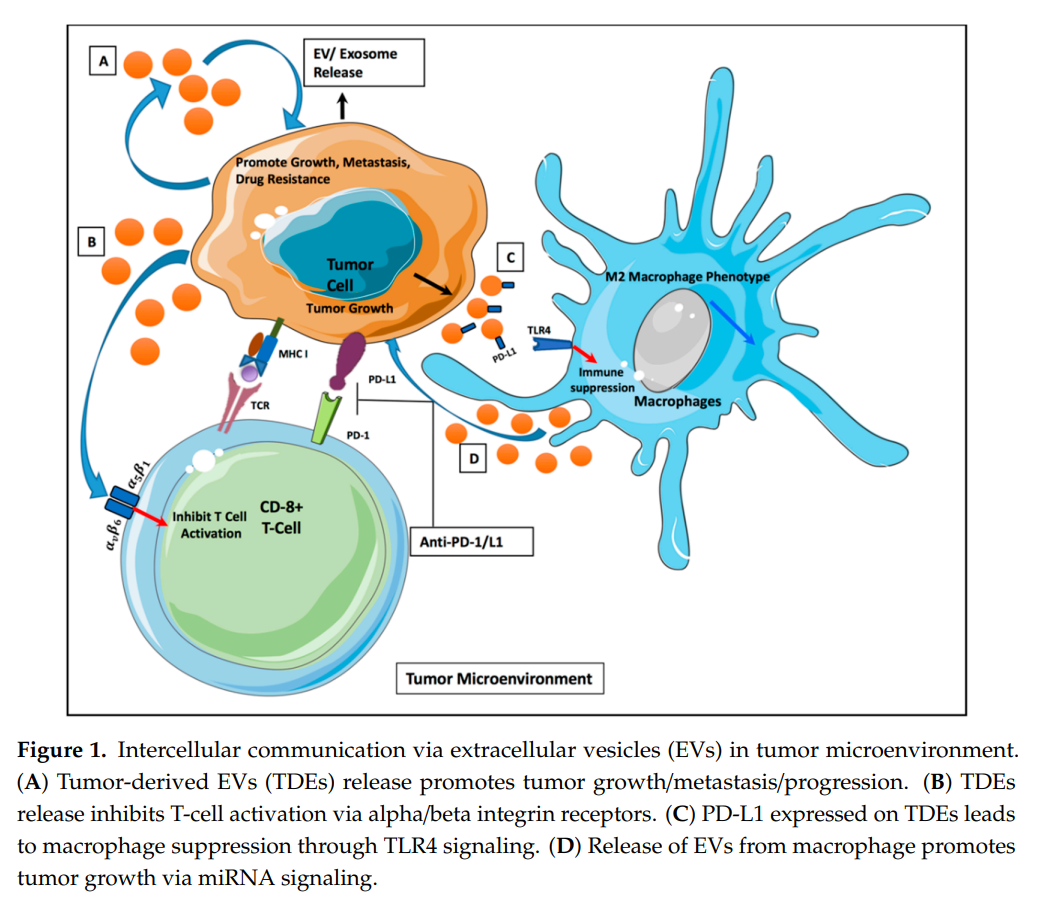
Unlike non-transformed cells, cancer cells have a unique metabolism that produces enough biomaterials and energy for endless proliferation. Furthermore, metastasis, cell death resistance, and other malignant characteristics are all influenced by reprogrammed metabolism. According to Warburg's 1956 report, an essential component of cancer metabolism is accelerated glycolysis [Warburg, 1956]. High glycolytic activity provides biomolecule precursors for cellular processes and structure. In addition to replenishing NAD+ for glycolysis, lowering pH for invasion, and inducing immune escape, lactate production in glycolysis plays a significant role in the progression of malignancies. Pyruvate is converted to lactate by the enzyme lactate dehydrogenase A (LDHA), whose aberrant expression and activation have been linked to a number of cancers [Sheng et al., 2012; Le et al., 2010; Fantin et al., 2006]. So, it has been thought that LDHA represents a promising target for the prevention and treatment of cancer. The high expression profile and activated status of LDHA in many cancers are attributed to various mechanisms involving almost every stage of gene expression regulation.



The shorter arm of chromosome 11, which is a methylation hotspot, contains the LDHA gene. Numerous studies have demonstrated that LDHA expression was inhibited by DNA methylation in the promoter. When compared to IDH wild type, gliomas with mutant isocitrate dehydrogenase (IDH) had lower levels of LDHA, and the molecular basis for this finding may be that mutant IDH causes higher levels of methylation in the LDHA promoter. Additionally, according to Maekawa the retinoblastoma cell line NCC-RbC-51 hardly expresses LDHA, which is however restored by the demethylating agent 5aza-2′-deoxycytidine. All of these point to the crucial role that methylation modification plays in the mechanisms that activate LDHA. Because the promoter region of LDHA contains numerous elements for different transcription factors to bind to, a wide variety of transcription factors may regulate LDHA. Enhancing LDHA promotes a variety of malignant bio characteristics in cancers.

**Exosomes or microvesicles role in cancer Biomarker:**

Exosomal markers are emerging as intriguing targets for cancer detection because it has been suggested that exosome secretion is increased in cancer patients [Shah et al., 2018; Caivano et al., 2015; Cappello et al., 2017]. To achieve this, studies have examined the connection between plasma exosome levels and tumor burden [Theodorak et al., 2019; Osti et al., 2019; Logozzi et al., 2019; Giampieri et al., 2019]. The levels of CD63-positive and caveolin 1 (CAV1)-positive exosomes were upregulated in patients and associated with a worse prognosis, according to a study on stage IV oral SCC patients [Rodríguez et al., 2019]. Higher levels of exosomal CD81 and prostate-specific antigen were found in prostate cancer, which could be used to separate prostate cancer patients from those with benign prostatic hyperplasia and healthy individuals [Logozzi et al., 2019].



Intercellular communication via extracellular vesicles (EVs) in tumor microenvironment. (A) Tumor-derived EVs (TDEs) release promotes tumor growth/metastasis/progression. (B) TDEs release inhibits T-cell activation via alpha/beta integrin receptors. (C) PD-L1 expressed on TDEs leads to macrophage suppression through TLR4 signaling. (D) Release of EVs from macrophage promotes tumor growth via miRNA signaling.

The development of cancer and immune responses have been reported to depend on tumor-derived exosome proteins both functionally and prognostically [Costa-Silva et al., 2015; Peinado et al., 2012]. The majority of mass spectrometry analyses are used to identify various proteins in blood exosomes obtained from cancer patients and healthy individuals. Multiple distinct biomarker candidates were discovered after research on the surface proteins of exosomes from pancreatic ductal adenocarcinoma (CLDN4, EPCAM, CD151, LGALS3BP, HIST2H2BE, and HIST2H2BF) [Castillo et al., 2018]. One of the most extensively studied surface markers of exosomes derived from colon and pancreatic cancer is glipican 1 (GPC1) [Melo et al., 2015]. It has been demonstrated that patients with early- and latest-stage pancreatic cancer can be distinguished from healthy individuals and patients with benign pancreatic disease by the amount of GPC1+ circulating exosomes. The levels of GPC1+ exosomes are related to the tumor burden and post-operative patient survival, making it possible to detect pancreatic cancer and possibly assess treatment response [Melo et al., 2015]. According to some studies, serum CA19-9 and GPC1+ exosomes may work as a diagnostic marker for pancreatic cancer [Buscail et al., 2019; Xiao et al., 2020].

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