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Full Length Research Paper

Clinical pathology and their potential application in disease diagnosis

Vikash Kumar*[1], Suvra Roy[2], Debtanu Barman[4], Lokesh paul[3] and Kundan Kumar[1]

[1]Aquatic Environment and Health Management Division, Central Institute of Fisheries Education, Mumbai, India, 400061. [2]Fish Genetics and Biotechnology Division, Central Institute of Fisheries Education, Mumbai, India, 400061. [3]Aquaculture division, Central Institute of Fisheries Education, Mumbai, India, 400061. [4]Center for Aquaculture Research & Development (CARD), St. Xavier's Vocational Training Center, Don Bosco, Bishramganj, Tripura, India, 799103.

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Clinical pathology is collection of sample for analytical work performed in a clinical laboratory through specific pathology fields and it includes clinical chemistry, hematology and microbiology. It consists of simple blood test, microbiological tests as well as other fluid tests to detect, diagnose and monitor various disease related conditions. Entire procedure may involve a routine health check, a diagnostic test aimed at detecting a particular condition or disease and follow-up tests to evaluate effectiveness of the treatment for a condition, which are often a series of easy to administer, convenient tests conducted at special laboratories to deliver quick and fairly accurate results.

Key words: Clinical pathology, Haematology, microbiology, clinical chemistry, disease.

INTRODUCTION

Fish exhibit both specific and non-specific defence mechanism against disease. Non-specific defence includes skin, scales as well as the mucus layer secreted by the epidermis that traps microorganism and inhibits their growth.

If pathogens breach this defence, fish can develop inflammatory response that increase the flow of blood to infected areas and deliver white blood cells that attempt to destroy the pathogens (Janeway and Medzitov, 2002).

Specific defence is specialized response to particular pathogen recognized by the fish body that is adaptive immune responses (Aoki et al., 2008). The word pathology is often associated with the term disease which is the combination of two words dis + ease meaning discomfort.

The word pathology is derived from two Greek words, viz. 'patho' and 'logos' meaning feeling or suffering and study respectively together stands for "Study of disease". "Pathology is the branch of science in which we study the nature, cause and effect of disease." It deals with all aspects of a disease or condition, focusing particularly on the nature, cause, and development of certain abnormal conditions, additionally on functional and structural changes that occur as a result of a disease (Robbins, 2010).

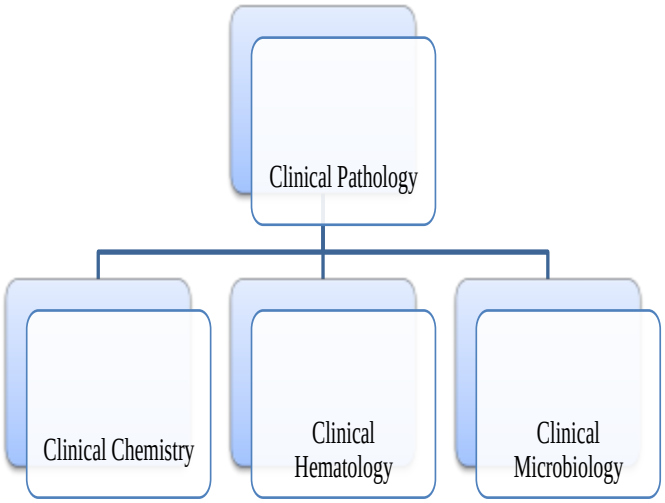
*Corresponding author. E-mail: kumar.vika.vikash2@gmail.com

Clinical Pathology

Clinical pathology is a branch of pathology which deals with the diagnosis of disease based on the laboratory analysis of bodily fluids such as blood, urine and tissues using the tools of chemistry, microbiology, hematology and molecular pathology and in other word it is the study and diagnosis of diseases by analyzing body fluids. The importance of these disciplines in modern pathology is emphasized by various statistics showing that 85% of diagnosis is made on the basis of pathology testing.

Disease diagnosis is done by correlating the signs and symptoms of the animal (clinical features) with the information given by the standards and the lab investigation reports. In certain situations, there may be some difficulty in making a diagnosis such as number of diseases having almost similar clinical features, rarely occurring or newly -

A clinical pathology is mainly of three types:



1. Clinical chemistry
2. Clinical Hematology
3. Clinical Microbiology

emerged easily un-identifiable disease, especially by a general pathologist. Under such circumstances, a specialist opinion may be required.

Clinical chemistry

Clinical chemistry (also known as chemical pathology, clinical biochemistry or medical biochemistry) is an area of clinical pathology, concerned with analysis of body fluids mainly hormones, enzymes, immune system and antibody and the function of drugs on the system, or pharmacology.

Clinical enzymes

Enzymes are biological catalysts regulating metabolic reactions and are globular proteins with a specific tertiary shape. The part of the enzyme that acts as a catalyst is called the active site.

The remaining part is much larger and is involved in maintaining its specific shape. When a reaction involving an enzyme occurs, a substrate is turned into a product which can be of one or more molecules. The active site of an enzyme is complementary to the substrate is catalyses.

The presence of enzymes in serum indicates occurrence of tissue or cellular damage resulting in release of intracellular components in blood.

The measurement of the serum levels of numerous enzymes is of diagnostic significance such as the purpose of an assay for liver enzyme is to ascertain the potential for liver cell damage. Enzyme activity is expressed in International unit (IU) corresponding to the amount of enzyme that catalyzes the conversion of one micromole (μmol) of substrate to product per minute. Commonly assayed enzymes are:

Alanine transaminase, **ALT** (sometimes referred as serum glutamate-pyruvate transaminase, SGPT)

Mode of action

- ALT is a cytoplasmic enzyme
- Catalyzes the reversible deamination of alanine to pyruvate.

Alanine —ALT→ **Pyruvate**

α-ketoglutarate —ALT→ **Glutamate**

- Major cellular sources of increased serum enzyme activity:
  - -Hepatocytes
  - -Skeletal myocytes

Clinical significance

It is commonly measured clinically as a part of diagnostic evaluation of hepatic-cellular injury, to determine liver health. When used in diagnostics, it is almost always measured in international units/liter (U/L). While sources vary on specific normal range values for patients, 10-40 U/L is the standard normal range for experimental studies. Alanine transaminase shows a marked diurnal variation.

Aspartate transaminase, **AST** (also referred to as serum glutamate-oxaloacetate transaminase, SGOT)

Mode of action

- Catalyzes the transamination of L-aspartate to oxaloacetate and oxo-glutarate to glutamate.

Aspartate —AST→ **oxaloacetate**

α-keto glutarate —AST→ **glutamate**

- Pyridoxal-5'-phosphate functions as the prosthetic group.
- Normal level of AST in serum is 0-41 IU/L.

Clinical significance

1. Physiological changes in catalytic concentration of AST in serum. AST is higher in normal newborns.
2. Pathological changes in catalytic concentration of

AST in serum

A) Increased values are found in:

- Myocardial infarction
- Liver disease
- Pancreatic disease
- Trauma

B) Decreased values are found in:

- Beri beri
- uncontrolled diabetes mellitus with acidosis

The typical liver enzymes measured are AST and ALT. ALT is particularly diagnostic of liver involvement as this enzyme is found predominantly in hepatocytes. When assaying for both ALT and AST, the ratio of the level of these two can also be diagnosed. Normally in liver disease or damage that is not of viral origin the ratio of ALT/AST is less than 1. However, with viral hepatitis the ALT/AST ratio will be greater than 1. Measurement of AST is useful not only for liver involvement but also for heart disease or damage. The level of AST elevation in serum is directly proportional to the number of cells involved as well as on the time following injury that the AST assay was performed. Following injury, level of AST rises within 8 hours and peaks 24–36 hours later. Within 3–7 days the level of AST should return to pre-injury level, provided a continuous insult is not present or further injury occurs.

Lactate Dehydrogenase (LDH)

Lactate dehydrogenase (LDH or LD) is an enzyme present in a wide variety of organisms, including plants and animals. Lactate dehydrogenases exist in four distinct enzyme classes; two cytochrome c-dependent enzymes, each acting on either D-lactate or L-lactate and two NAD (P)-dependent enzymes, each acting on either D-lactate or L-lactate.

Lactate dehydrogenase catalyzes the inter-conversion of pyruvate and lactate with concomitant inter-conversion of NADH and NAD^+. It converts pyruvate, the final product of glycolysis, to lactate when oxygen is absent or in short supply and it performs the reverse reaction during the Cori cycle in the liver. At high concentrations of lactate, the enzyme exhibits feedback inhibition, and the rate of conversion of pyruvate to lactate is decreased. It also catalyzes the dehydrogenation of 2-Hydroxybutyrate, but it is a much poorer substrate than lactate.

LDH is elevated in myocardial infarction, blood disorders. It is a tetrameric protein and made of two types of subunits namely H = Heart, M = skeletal muscle. It exists as 5 different isoenzymes with various combinations of H and M subunits.

Mode of action

Lactate ⟹ **Pyruvate**

NAD ⟹ **NADH**

- Major cellular sources of increased serum LDH activity:

Hepatocytes, skeletal myocytes, Erythrocytes, cardiac myocytes

Clinical significance:

Increased serum LDH activity is a marker of

✓ Hepatocyte damage, muscle damage and hemolysis.

✓ Can be due to reversible/ irreversible, focal or diffuse cell damage.

- Normal value in serum is 60-200 IU/ L and the measurement of LDH is especially diagnostic for myocardial infarction.

Alkaline Phosphatase (ALP)

ALP is a group of enzymes that have maximal activity at a high pH 9.0-10.5 and widely distributed throughout the body. High levels are seen in liver, bone, placenta and intestine and useful to assess hepatobiliary and bone diseases.

In hepatobiliary obstruction, hepatocytes lining the biliary ducts induce the ALP synthesis. A high level of ALP is indicative of extrahepatic obstruction rather than intrahepatic obstruction. In bones, the enzyme is derived from osteoblasts and hence increases in bone diseases like rickets, osteomalacia, neoplastic diseases with bone metastates and healing fractures.

The activity of the bone isoenzyme can be estimated by heat treating a serum sample at 56^oC. The bone ALP is heat liable and is destroyed or heat inactivated at this temperature. Measurement of ALP before and after heat treatment gives a measure of bone ALP.

Mode of action

- Hydrolyse much type of phosphate esters, catalyze the dephosphorylation of ATP, requires Mg^+ as activator.
- Maximal activity at pH 9.0-10.5.
- Major cellular sources of increased serum ALP activity:

Hepatocytes, osteoblasts, biliary epithelium.

Clinical significance

Cholestatic liver disease, osteosarcoma, pancreatic carcinoma, pancreatitis, hepatic necrosis, induction of drugs/hormones.

- Normal level of ALP in serum is 10-13 KA units/100 ml.

Creatine Kinase (CK)

Measurement of the activity of creatine kinase in the blood, analysis of a muscle biopsy, and recordings from an electromyograph frequently establishes that the muscle weakness is due to primary degeneration of the muscles. Creatine kinase is an enzyme of muscle fibres released into the bloodstream when the fibres degenerate, as in the muscular dystrophies.

Creatine kinase is a dimer made of 2 monomers occurring in the tissue. Skeletal muscle contains M subunit, Brain contains B subunits. Three different isoenzymes are formed.

Creatine + ATP $\rightleftharpoons$ phosphocreatine + ADP

(Phosphocreatine – serves as energy reserve during muscle contraction)

Clinical Hematology

Clinical hematology is mainly the study and analysis of blood either examined as a "whole," as plasma (the fluid left when red and white blood cells are removed), or as serum (a clear fluid that separates from blood when it clots).

Blood

Blood transports oxygen and carbon-di-oxide, nutrients, hormones, metabolic waste to kidneys, antibodies and several other things throughout the body. It contains living cells namely Red Blood Corpuscles (transports oxygen and carbon-di-oxide), White Blood Corpuscles (nourishment and cleaning of body) and Platelets (blood clotting post injury).

Approximately 55 percent of blood is plasma, a straw-colored clear liquid which carries the solid cells and the platelets (Franklin Institute, 2009)

Composition of blood

- 55% plasma
- 45% blood cells

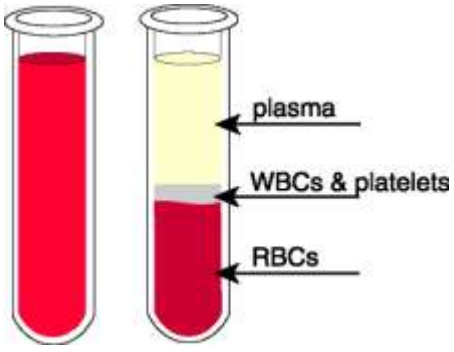


Figure. Fish blood component**.**

Function

Blood supply oxygen to tissues, nutrients such as glucose, amino acids, and fatty acids (dissolved in the blood or bound to plasma proteins example blood lipids), removes waste such as carbon dioxide, urea, and lactic acid, immunizes body through circulation of white blood cells, and detection of foreign material by antibodies, coagulates blood during bleeding as body's self-repair mechanism act as messenger for transport of hormones and the signaling of tissue damage, regulates body pH (the normal pH of blood is in the range of 7.35–7.45), core body temperature and hydraulic functions (Spiders, 2007)

Plasma

Plasma is the liquid portion of blood which serves as a transport medium for delivering nutrients to the cells of the various organs, transporting metabolic wastes to the kidneys, liver, and lungs for excretion and transporting blood cells. It plays a critical role in maintaining normal blood pressure, distribution of heat throughout the body and maintaining homeostasis (Dennis, 1999).

Plasma is derived when all the blood cells—red blood cells (erythrocytes), white blood cells (leukocytes), and platelets (thrombocytes)—are separated from whole blood. The remaining straw-coloured fluid is 90–92 percent water containing critical solutes necessary for sustaining health and life. Important constituents include electrolytes such as sodium, potassium, chloride, bicarbonate, magnesium, and calcium. In addition, there are trace amounts of other substances, including amino acids, vitamins, organic acids, pigments, and enzymes. Hormones such as insulin, corticosteroids, and thyroxine are secreted into the blood by the endocrine system. Plasma concentrations of hormones must be carefully regulated for good health. Nitrogenous wastes (example, urea and creatinine) transported to the kidney for excretion increase marked with renal failure (Tuskegee University, 2013).

Plasma contains 6–8 percent proteins. One critical group is the coagulation proteins and their inhibitors, synthesized primarily in the liver. Coagulation inhibitor proteins help to prevent abnormal coagulation (hyper-coagulability) and to resolve clots after they are formed. When plasma is allowed to clot, fibrinogen converts to fibrin, trapping the cellular elements of blood. The resulting liquid, devoid of cells and fibrinogen, is called serum. Biochemical testing of plasma and serum is an important part of modern clinical diagnosis and treatment monitoring. High or low concentrations of glucose in the plasma or serum help to confirm serious disorders such as diabetes mellitus and hypoglycemia. Substances secreted into the plasma by cancers may indicate an occult malignancy; for instance, an increased concentration of prostate-specific antigen (PSA) in a middle-aged asymp-

tomatic man may indicate undiagnosed prostate cancer.

Serum albumin, another protein synthesized by the liver, constitutes approximately 60 percent of all of the plasma proteins. It is very important in maintaining osmotic pressure in the blood vessels; it is also an important carrier protein for a number of substances, including hormones. Other proteins called alpha and beta globulins transport lipids such as cholesterol as well as steroids, sugar, and iron. The gamma globulins, or immunoglobulin's, are an important class of proteins that are secreted by B lymphocytes of the immune system. They include most of the body's supply of protective antibodies produced in response to specific viral or bacterial antigens. Cytokines are proteins synthesized by cells of various organs and cells found in the immune system and bone marrow in order to maintain normal blood cell formation (hematopoiesis) and regulate inflammation.

The electrolytes and acid-base system found in the plasma are finely regulated. For example, potassium is normally present in plasma in a concentration of only 4 milli-equivalents per litre. A slight rise in plasma potassium (to 6–7 milli-equivalents per litre) can result in death. Likewise, sodium, chloride, bicarbonate, calcium, and magnesium levels in the plasma must be precisely maintained within a narrow range. Smaller molecules such as sodium, potassium, glucose, and calcium are primarily responsible for the concentration of dissolved particles in the plasma. However, it is the concentration of much larger proteins (especially albumin) on either side of semi-permeable membranes such as the endothelial cells lining the capillaries that creates crucial pressure gradients necessary to maintain the correct amount of water within the intravascular compartment and, therefore, to regulate the volume of circulating blood. So, for example, patients who have kidney dysfunction or low plasma protein concentrations (especially low albumin) may develop a migration of water from the vascular space into the tissue spaces, causing edema (swelling) and congestion in the extremities and vital organs, including the lungs.

Blood corpuscles

Blood corpuscles are of following three types

1. Erythrocyte
2. Leucocyte
3. Thrombocyte

Erythrocytes

Red blood cells, also called erythrocytes are responsible for its characteristic colour and carrying oxygen from the lungs to the tissues. The mature fish red blood cell is small, round, and biconcave; it appears dumbbell-shaped in profile. The cell is flexible and assumes a bell shape as it passes through extremely small blood vessels. It is covered with a membrane composed of lipids and proteins, a nucleus and contains hemoglobin, a red iron-rich protein that binds oxygen (Snyder and Sheafor, 1999).

The function of the red cell and its hemoglobin is to carry oxygen from the lungs or gills to all the body tissues and to carry carbon dioxide, a waste product of metabolism, to the lungs, where it is excreted. In invertebrates, oxygen-carrying pigment is carried free in the plasma.

- Human blood - 4.4 to 5 million/mm^3
- Fish blood- 1.05 to 3 million/mm^3

Low total count of RBC leads to anemia and abnormal rise is known as polycythemia. Decrease in the number of RBC is called erythrocytopenia which causes O_2 shortage in blood and tissue. It is important to note that oxygen shortage stimulates the kidney to secrete a hormone called erythropoietin into the blood. Erythropoietin stimulates the bone marrow to increase the production of RBCs.

Formation

The red cell develops in kidney in several stages: from a hemocytoblast, a multi-potential cell in the mesenchyme, it becomes an erythroblast (normoblast); during two to five days of development, the erythroblast gradually fills with hemoglobin. In a late stage the cell is called areticulocyte, which ultimately becomes a fully mature red cell. Formation of erythrocytes is called as erythropoiesis. In the early few weeks of embryonic life, primitive nucleated RBCs are produced in the yolk sac.

- Human –Bone marrow, liver and spleen.
- Fish – Anterior kidney, liver and spleen.

Iron and protein are the necessary raw materials for the synthesis of hemoglobin. However vitamin B12 and folic acid - maturation of RBCs. Deficiency causes anemia. Excess RBCs are stored in spleen.

Leukocytes

White blood cells, also called leukocytes or white corpuscles, are cellular component of the blood that lacks hemoglobin, has a nucleus, is capable of motility, and defends the body against infection and disease by ingesting foreign materials and cellular debris, by destroying infectious agents and cancer cells, or by producing antibodies. A healthy adult human has between 4,500 and 11,000 white blood cells per cubic millimeter of blood. Fluctuations in white cell number occur during the day; lower values are obtained during rest and higher values during exercise. Intense physical exertion may cause the count to exceed 20,000 per cubic

millimeter. White cell count also may increase in response to convulsions, pain and certain disease states, such as infections and intoxications (LaFleur-Brooks, 2008).

Although white cells are found in the circulation, most occur outside the circulation, within tissues, where they fight infections; the few in the bloodstream are in transit from one site to another. As living cells, their survival depends on their continuous production of energy. The chemical pathways utilized are more complex than those of red blood cells and are similar to those of other tissue cells. White cells, containing a nucleus and are able to produce ribonucleic acid (RNA), can synthesize protein. White cells are highly differentiated for their specialized functions, and they do not undergo cell division (mitosis) in the bloodstream; however, some retain the capability of mitosis. On the basis of their appearance under a light microscope, white cells are grouped into three major classes lymphocytes, granulocytes and monocytes each of which carries out somewhat different functions.

- Human - 5,000 to 10,000/mm^3
- Fish - 90,000-1,50,000/mm^3

When the number of WBCs in your blood increases, this is a sign of an infection somewhere in your body. They are broadly classified in to two types and they are

- Agranulocytes
- Granulocytes

Agranulocytes

Agranulocytes are white blood cells in which there is no granule in the cytoplasm. Two different types of agranulocytes are available namely lymphocytes and monocytes. Normally the human blood has 3 types of lymphocytes namely B cells, T cells and NK cells. Lymphocytes are commonly found in lymphatic system of the body in particular the NK (natural killer) cells. Each type of cells has different set of function. B cells are responsible for binding the pathogens that make up antibodies in our body. T cells trigger the immunity system and takes control of immunity level when the body metabolism is affected. NK cells do the work of destroying the cells which are infected by virus. Among these, T cells are highly important because they not only control the immune system but also remember the earlier infections and diseases caused by invaders.

Lymphocytes

Lymphocytes contain scant cytoplasm with large rounded nucleus and non-phagocytic. Lymphocytes, further divided into B and T cells, are responsible for the specific recognition of foreign agents and their subsequent removal from the host. B lymphocytes secrete antibodies, which are proteins that bind to foreign microorganisms in body tissues and mediate their destruction. Typically, T cells recognize virally infected or cancerous cells and destroy them, or they serve as helper cells to assist the production of antibody by B cells. Also included in this group are natural killer (NK) cells, so named for their inherent ability to kill a variety of target cells. In a healthy person, about 25 to 33 percent of white blood cells are lymphocytes.

- Human: 20-45%
- Fish: 70-90%

Monocytes

They are the largest of all type of leucocytes and somewhat amoeboid in shape. They have much cytoplasm with bean shaped nucleus. They are motile, and engulf bacteria and cellular debris through phagocytosis. Generally they changes into macrophages after entering tissue spaces. The monocyte of fishes forms about 0.1% of the circulating leukocyte population. Monocytes move from blood to sites of infection and differentiate further into macrophages. These cells are scavengers that phagocytose whole or killed microorganisms and are therefore effective at direct destruction of pathogens and cleanup of cellular debris from sites of infection. Neutrophils and macrophages are the main phagocytic cells of the body; macrophages being larger and longer-lived than neutrophils. Some macrophages are important as antigen-presenting cells, which phagocytose and degrade microbes and present portions of these organisms to T lymphocytes, thereby activating the specific acquired immune response.

- Human: 2-10%
- Fish: 0.1%

Granulocytes

Granulocytes, the most numerous of the white cells, recognize the body of larger pathogenic organisms like protozoans or helminths and are also key mediators of allergy and other forms of inflammation. These cells contain many cytoplasmic granules, or secretory vesicles, that harbour potent chemicals important in immune responses.

They also have multilobed nuclei, and because of this they are often called polymorphonuclear cells. On the basis of how their granules take up dye in the laboratory, granulocytes are subdivided into three categories: neutrophils, eosinophils, and basophils. The most numerous of the granulocytes—making up 20-30 percent of all white cells—are neutrophils.

They are often one of the first cell types to arrive at a site of infection, where they engulf and destroy the infectious microorganisms through a process called phagocytosis. Eosinophils and basophils, as well as the tissue cells called

mast cells, typically arrive later. The granules of basophils and of the closely related mast cells contain a number of chemicals, including histamine and leukotrienes that are important in inducing allergic inflammatory responses. Eosinophils destroy parasites and also help to modulate inflammatory responses.

Eosinophils

Eosinophils, a type of leukocyte is characterized histologically by its ability to be stained by acidic dyes (e.g., eosin) and functionally by its role in mediating certain types of allergic reactions. They contain large granules, and the nucleus exists as two non-segmented lobes. In addition, the granules of eosinophils typically stain red, which makes them easily distinguished from other granulocytes when viewed on prepared slides under a microscope. Eosinophils are rare, making up less than 1 percent of the total number of white blood cells occurring in the human body.

Eosinophils, being produced in the bone marrow are released into the circulation. They leave the circulation within hours of release from the marrow and migrate into the tissues (usually those of the skin, lung, and respiratory tract) through lymphatic channels. Similar to neutrophils, eosinophils respond to chemotactic signals released at the site of cell destruction. These chemical signals orient eosinophils to migrate in the direction of cell damage. Eosinophils are actively motile and phagocytic and participate in hypersensitivity and inflammatory reactions, primarily by dampening their destructive effects.

Eosinophils are also involved in defense against parasites. Eosinophils and antibodies of the immunoglobulin E (IgE) class work together to destroy parasites such as the flatworms that causes chistosomiasis. The eosinophils plaster themselves to the worms bound to IgE and release chemicals from their granules that break down the parasite's tough, protective skin.

- Human: 1-6%
- Fish : 1-3%

Basophils

Basophil, a type of leukocyte is characterized histologically by its ability to be stained by basic dyes and functionally by its role in mediating hypersensitivity reactions of the immune system.

Basophils are the least numerous of the granulocytes and account for less than 1 percent of all white blood cells occurring in the human body. Their large granules stain purple-black in colour and almost completely obscure the underlying double-lobed nucleus. Within hours of their release from the bone marrow, basophils migrate from the circulation to the barrier tissues (e.g., the skin and mucosa), where they synthesize and store histamine, a natural modulator of the inflammatory response. When antibodies of the immunoglobulin E (IgE) class bind to specialized receptor molecules on basophils, the cells release their stores of inflammatory chemicals, including histamine, serotonin, and leukotrienes. These chemicals have a number of effects, including constriction of the smooth muscles, which leads to breathing difficulty; dilation of blood vessels, causing skin flush and hives; and an increase in vascular permeability, resulting in swelling and a decrease in blood pressure. Basophils also incite immediate hypersensitivity reactions in association with platelets, macrophages, and neutrophils.

- Human: 0-1%
- Fish: 0-1%

Neutrophils

In all animals, neutrophils are the most numerous and important components of host defence against bacteria, viruses and fungal infections (Smith and Lumsden, 1983). In fish, the anterior kidney is the central immune organ where neutrophils are being produced (Bols et al., 2001), however they can be found in spleen and blood, and are commonly increased in inflammatory lesions (Afonso et al., 1998). The granules of neutrophils typically stain pink or purple-blue following treatment with a dye. About 20-30 % of all the white bloods cells occurring in the human body are neutrophils.

The neutrophils are fairly uniform in size with a diameter between 12 and 15 micrometers. The nucleus consists of two to five lobes joined together by hair like filaments. Neutrophils move with amoeboid motion. They extend long projections called pseudopodium into which their granules flow; this action is followed by contraction of filaments based in the cytoplasm, which draws the nucleus and rear of the cell forward. In this way neutrophils rapidly advance along a surface. It takes about one week to form mature neutrophils from a precursor cell in the marrow; yet, once in the blood, the mature cells live only a few hours or perhaps a little longer after migrating to the tissues. To guard against rapid depletion of the short-lived neutrophils (for example, during infection), the bone marrow holds a large number of them in reserve to be mobilized in response to inflammation or infection. Within the body the neutrophils migrate to areas of infection through chemotaxis and are attributed to substances liberated at sites of tissue damage. Among 100 billion neutrophils circulating outside the bone marrow, half are in the tissues and rest in the blood vessels; of those in the blood vessels, half are within the mainstream of rapidly circulating blood and remaining move slowly along the inner walls of the blood vessels -

(marginal pool), ready to enter tissues on receiving a chemotactic signal from them.

Neutrophils are actively phagocytic. The granules of neutrophils are microscopic packets of potent enzymes capable of digesting many types of cellular materials. When a bacterium is engulfed by neutrophils, it is encased in a vacuole lined by the invaginated membrane. The granules discharge their contents into the vacuole containing the organism.

As this occurs, the granules of the neutrophils are depleted (degranulation). A metabolic process within the granules produces hydrogen peroxide and a highly active form of oxygen (superoxide), destroy the ingested bacteria. Final digestion of the invading organism is accomplished by enzymes.

An abnormally high number of neutrophils circulating in the blood is called neutrophilia. It is typically associated with acute inflammation, though it may result from chronic myelogenous leukemia, a cancer of the blood-forming tissues.

An abnormally low number of neutrophils is called neutropenia which can be caused by various inherited disorders affecting the immune system as well as by a number of acquired diseases, including certain disorders that arise from exposure to harmful chemicals. Neutropenia significantly increases the risk of life-threatening bacterial infection.

- Human: 40-75%
- Fish: 20-30%

Thrombocyte

Platelets, also called thrombocyte are colourless, non-nucleated blood component is important in the formation of blood clots (coagulation). Platelets are found only in the blood of mammals.

They are formed when cytoplasmic fragments of megakaryocytes, which are very large cells in the bone marrow, pinch off into the circulation as they age and stored in the spleen. Some evidence suggests platelets may also be produced or stored in the lungs, where megakaryocytes are frequently found.

Platelets play an important role in the formation of a blood clot by aggregating to block a cut blood vessel and provide a surface on which strands of fibrin form an organized clot, by contracting to pull the fibrin strands together to make the clot firm and permanent and perhaps most important, by providing or mediating a series of clotting factors necessary to the formation of the clot.

Platelets also store and transport several chemicals, including serotonin, epinephrine, histamine and thromboxane; upon activation these molecules are released and initiate local blood vessel constriction, which facilitates clot formation (Schmaier et al., 2011).

Typically they are elongated cells, often termed spindle cells having densely staining nuclei, surrounded by a minute amount of cytoplasm. It is this spent thrombocyte which has been frequently confused with the lymphocyte. When observed in the living state by phase-contrast microscopy, a retractile vacuole can be seen at the base of the pointed end of the thrombocyte, just anterior to the nucleus.

- Human: 250000/mm^3
- Fish: 60000-70000/mm^3

Types of Blood Disorders

Blood disorders can affect any of the three main compon ents of blood:

- Red blood cells, which carry oxygen to the body's tissues
- White blood cells, which fight infections
- Thrombocytes, which help blood to clot

Blood disorders can also affect the liquid portion of blood, called plasma.

Treatments and prognosis for blood diseases vary, depending on the blood condition and its severity.

Disease related to plasma

The most well-known disease related to plasma is hemophilia. An inherited change in one of the clotting proteins (called factor VIII) leaves it dysfunctional. This single change disrupts the entire sequence of chemical reactions necessary for clotting. As a result, people with hemophilia can suffer severe swelling, bruising and bleeding from simple day to day events that the rest of the population take for granted.

Disease related to RBC

Blood diseases involving the red blood cells include:

- Anemia (or anaemias) is disease characterized by low oxygen transport capacity of the blood, due to low red cell count or some abnormality in red blood cells or hemoglobin.
  - Iron deficiency anemia is the most common anemia, occurs when the dietary intake or absorption of iron is insufficient, and hemoglobin, which contains iron, cannot be formed.
  - Sickle-cell disease is a genetic disease that results in abnormal hemoglobin molecules. When these release their oxygen load in the tissues, they become insoluble, leading to mis-shaped red blood cells. These sickle shaped red cells are less deformable and viscoelastic meaning that they have become rigid and can cause blood vessel blockage, pain, strokes, and other tissue damage.
  - Thalassemia is a genetic disease that results in the production of an abnormal ratio of hemoglobin subunits.

- Spherocytosis is a genetic disease that causes a defect in the red blood cells cytoskeleton, causing the red blood cells to be small, sphere-shaped, and fragile instead of donut-shaped and flexible.
- Pernicious anemia is an autoimmune disease wherein the body lacks intrinsic factor, required to absorb vitamin B_{12} from food. Vitamin B_{12} is needed for the production of hemoglobin.
- Aplastic anemia is caused by the inability of the bone marrow to produce blood cells.
- Pure red cell aplasia is caused by the inability of the bone marrow to produce only red blood cells.

- Hemolysis is the general term for excessive breakdown of red blood cells. It may have several causes and can result in hemolytic anemia.
- The malaria parasite spends part of its life-cycle in red blood cells, feeds on their hemoglobin and then breaks them apart, causing fever. Both sickle-cell disease and thalassemia are more common in malaria areas, because these mutations convey some protection against the parasite.
- Polycythemias (or erythrocytoses) are diseases characterized by a surplus of red blood cells. The increased viscosity of the blood can cause a number of symptoms.
  - In polycythemia vera the increased number of red blood cells results from an abnormality in the bone marrow. (Merriam-webster, 2009; MedicineNet.com, 2000)

Disease related to WBC

White cells defend against disease, which explains why their number increases in the bloodstream when the body is under infectious assault. There are some diseases of the blood and blood-forming organs themselves that can increase the white count. Disorders of the spleen, for example, can produce white cell abnormalities, because this organ is a major source of lymphocytes (cells responsible for making protective antibodies). Diseases of the bone marrow are likely to affect neutrophil production.

Leukemia

Leukemia, characterized by an abnormal increase in the number of white cells, is one of the most dangerous of blood disorders. The cancer like disease results from a severe disturbance in the functioning of the bone marrow. Chronic leukemia, which strikes mainly in middle age, produces an enormous increase in neutrophils, which tend to rush into the bloodstream at every stage of their development, whether mature or not. Patients with the chronic disease may survive for several years, with appropriate treatment.

In acute leukemia, more common among children than adults, the marrow produces monster-sized, cancerous-looking white cells. These cells not only crowd out other blood components from the circulation, they also leave little space for the marrow to produce the other elements, especially the red cells and platelets. Acute leukemia run their fatal course in a matter of weeks or months—although there have been dramatic instances of sudden remission. The cause is unknown, but recent evidence strongly suggests that a virus may be responsible for at least some forms of the disease (Mathers et al., 2001)

Disease related to Thrombocytes (Platelets)

Disorders of platelet number

Reduction in the number of blood platelets (thrombocytopenia) may be the result of impaired production or increased destruction of platelets. Normal platelet counts are between 60,000 to 70,000 per cubic millimetre. When the platelet count drops to 20,000 to 35,000 per cubic millimetre, and particularly to 10,000 to 20,000 per cubic millimetre, spontaneous bleeding may occur.

Thrombocytopenia is associated with such blood diseases as aplastic anemia and leukemia and is attributed to impaired production of platelets. Similarly, excessive radiation, exposure to certain chemicals (such as benzene), or drugs used in cancer chemotherapy decrease the production of platelets. In sensitive persons, drugs such as quinidine (used in the treatment of malaria) provoke platelet antibodies and platelet destruction, resulting in thrombocytopenia. Thrombocytopenia also may accompany certain infections such as measles and autoimmune disorders such as systemiclupus erythematosus and idiopathic thrombocytopenic purpura.

Thrombocytopenia, if sufficiently severe, is accompanied by spontaneous bleeding from the capillaries. This causes the appearance of tiny purplish spots (petechiae) or larger black-and-blue areas (ecchymoses) in the skin. Bleeding occurs commonly from the nose and gums and occasionally from sites such as the urinary tract and the intestine; hemorrhage in the brain can have serious consequences.

Clinical Microbiology

Clinical microbiology is a branch of microbiology which deals with the study of microorganisms including bacteria, viruses, fungi and parasites which are of medical importance and are capable of causing diseases. It includes the study of microbial pathogenesis and epidemiology and is related to the study of disease pathology and immunology. Clinical microbiology is also

known as medical microbiology. Clinical microbiology plays a crucial role in the health of individual and the communities in which they reside. Most microbes that live on or within the body are beneficial and help keep individuals healthy. Distinguishing between microorganisms that are beneficial and those that are disease-producing is a critical function of the clinical microbiologist. Clinical microbiology also impacts the health by helping to manage infectious disease outbreaks by identifying pathogens that could potentially infect dozens, hundreds, or even thousands of individuals (Prescott et al., 2005).

The primary task of the clinical microbiologist is to identify as quickly as possible any microorganism in a specimen that represents the possible causative agent of an infectious disease. Presumptive identification of microbes can be made by microscopically examining direct mounts of an appropriate portion of the specimen or thin smears that have been stained with one of a variety of dyes. Rapid presumptive diagnoses can also be made by directly testing specimens with a variety of immunological reagents.

The practice of general bacteriology still follows traditional culture methods that were introduced by Robert Koch before the turn of the century. Specimens are applied to the surface of a variety of agar culture media for the purpose of recovering in pure culture any bacterial species that may be clinically significant. Gram stains may determine the cellular morphology and staining characteristics of the bacteria, and a variety of rapid, direct tests can be performed to provide an early identification.

Bacterial identifications and antibiotic susceptibility tests may be performed in a variety of packaged systems. These systems typically use lyophilized, dried chemical substrates or antibiotics at various concentrations that are contained within microwells that are stamped into plastic polystyrene cards or trays. These cards or trays are placed in a 95°F (35°C) incubator after the microwells have been inoculated with a suspension of a pure culture of the bacterium to be identified.

The availability of monoclonal antibodies and nucleic acid probes, in which a highly specific portion of antigen or a small segment of nucleic acid can be tagged with either a fluorescent or an enzyme-linked detector, revolutionized the ability to detect specific microbes in biologic specimens and rapidly confirm the results of a culture.

The laboratory identification of fungi and the diagnosis of fungal infections are similar to that described for the bacteria. Specimens are inoculated on special fungal media, the plates are incubated for periods as long as 4 weeks, and the growth of any mold or yeast is identified morphologically and biochemically. Nucleic acid probes are available to quickly confirm any fungus colony suspected of being one of the dangerous pathogens. The laboratory identification of parasites involves detecting microscopically the typical forms in body fluids and secretions. Viruses can live only in viable cells and, for the most part, can survive briefly outside human or animal hosts. Therefore, culture techniques must use embryonated eggs, cell culture suspensions, thin cell sheets called monolayers, or laboratory animals. Species of viruses are identified by observing their ability to produce certain cytopathic effects in the cells where they are growing or to cause recognizable diseases in laboratory animals.

A clinical microbiology laboratory accepts specimens collected from a wide variety of body sites and through the use of various tools (example, microscopy, detection of microbial DNA and/or RNA, detection of microbial antigens, detection of antibodies, and/or growth of microorganisms in culture) can determine what microorganisms are present and which may be causing infection. The same laboratory takes painstaking care in detecting antimicrobial resistance mechanisms in the microorganisms recognized to aid physicians in selecting the most appropriate antimicrobial therapy (Nester et al., 2009).

Importance of clinical microbiology

Clinical microbiology is different from other sections of the laboratory because it deals with live microorganisms that multiply rapidly, are vulnerable to in appropriate handling between the specimen and the laboratory, are able to evolve resistance to therapeutic regimens, may or may not be causes of infections, and can disrupt the health through devastating local, regional, national, and worldwide outbreaks.

Clinical microbiology laboratories help to manage outbreaks of infectious disease by identifying and curbing pathogens that could potentially infect dozens, hundreds, or even thousands of animals. By communicating and coordinating with health laboratories, clinical microbiologists recognize, track, and control outbreaks at the community level. Should there be a bioterrorism event; clinical microbiologists would be the first line of protection by detecting the presence of a given disease in the community.

CONCLUSION

Clinical pathology is an important branch of pathology in which we examine body fluid such as serum, blood and based on the increase or decrease in these parameters in blood we provide a proper diagnosis for a disease. In clinical pathology we generally use tools like clinical chemistry, clinical hematology and clinical microbiology and these tools are very important for an accurate and effective diagnosis of disease. Accurate diagnostic tests

have a key role in disease management and the control of most infectious diseases. Unfortunately, in many developing countries, clinical care is often critically compromised by the lack of regulatory controls on the quality of these tests. The information available on the performance of a diagnostic test can be biased or flawed because of failings in the design of the studies which assessed the performance characteristics of the test. As a result, diagnostic tests are sold and used in much of the developing world without evidence of effectiveness. Misdiagnosis leading to failure to treat a serious infection or wasting expensive treatment on animal that are not infected remains a serious obstacle to health.

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