Investigating Transferrin: Deciphering its Complex Mechanisms in Cellular Iron Regulation and Implications in Health and Disease

ABSTRACT

Iron is a crucial constituent in cellular metabolism, playing a pivotal role in numerous enzymatic activities that are required for the maintenance of life. However, the lack of regulation in iron levels can lead to cellular damage through the Fenton reaction, which produces reactive oxygen species. Transferrin which is a glycoprotein functions crucially in contributing significantly to the movement of iron in biological systems. The polypeptide chain of transferrin, which is made of 700 amino acids, has a crucial role in iron binding and delivery. Transferrin has different N and C lobes that contribute to its exceptional attributes. In this paper, we looked at several aspects of transferrin which were explored, especially its diverse forms, characteristic structures, synthesis mechanisms, and metabolic functions. Various proteins, including lactoferrin, melanotransferrin, serum transferrin, and ovotransferrin, take part in regulating the transportation of iron and the prevention of iron homeostasis in vertebrates. We also explored the role of transferrin in various metabolic processes, which includes its activation of macrophages, antimicrobial attributes, and participation in immunological responses. A detailed assessment of the chemical attributes of transferrin provides useful information about its amino acid constituent, arrangement, and bonds with a broad spectrum of metal ions. This paper delves into taking part in scholarly reviews that address the therapeutic relevance of transferrin, stressing its function as a marker for diagnosing iron deficiency as well as its implications in health conditions such as hemochromatosis and atransferrinemia. This detailed assessment of transferrin that this paper presents makes a renowned development in how we understand its complex mechanisms,

thus enhancing how we comprehend iron regulations in cells and how it implies both health and disease.

1.0 Introduction

Iron is needed in many life forms to support their existence and enable them to grow as it takes part in several reactions catalysed by enzymes and metabolism in cells such as transport of electrons and oxygen, Biosynthesis of DNA and other nucleic acids, *etc.*).

In animals, iron is not found in their free naturally occurring state, however, through the Fenton reaction, excess iron catalyses the process of forming reactive oxygen species (or free radicals) from hydrogen peroxide (Naser, 2000; Neves, et al., 2009). Thus, causing harm to the structure of cells and eventual cell death (Crichton et al., 2002; van Campenhout et al., 2003). Normally, in the body, some mechanisms reduce the free iron present, thereby, curbing the harm resulting from iron toxicity. A non-toxic ferric form (Fe^{3+}) is produced when a transport or storage protein binds to unincorporated iron that should be a functional part of proteins (Ganz & Nemeth, 2006). In media where a living organism is found, substances that are complexed with the iron, contribute to dissolving it, conveying it within the animal and cellular delivery. In the living cells, iron is found in heme proteins such as myoglobin, cytochromes, and haemoglobin as a heme complex or as non-heme proteins such as ferritin, hemosiderin and **transferrin,** which are carried as a redox-inactive form (Naser, 2000). For the storage of iron, the non-heme protein (hemosiderin and ferritin) plays a major role, while the iron transport protein binds to an atom of iron and makes it inaccessible for the catalytic reaction involved in the formation of superoxide radical.

Transferrin is a glycoprotein one monomer and has a molecular weight of 80 kDa (kilo Dalton), nearly a length of 700 amino acids. It conveys iron that takes part in several processes of metabolism between the absorption sites (Hughes & Friedman, 2014), its storage

and utilization. It is therefore regarded as the principal iron-binding protein in the vertebrate species' plasma.

Transferrin is an iron-binding protein that binds iron reversibly, forming low-iron effects that inhibit bacteria pathogens from growing (Magnadottir, 2014).

2.0 A Brief Overview of Transferrins

Transferrins belong to a class of non-heme iron-binding glycoproteins broadly circulated in vertebrates' cells and body fluids. In the biological system, transferrins exist in several forms (Chung, 1984; de Jong, 1990) as follows:

- **The *serum transferrin*** also referred to as 'siderophilin', '13a metal-binding globulin' and 'serotransferrin', which is found in the serum of blood,
- **Lactoferrin** is also regarded as 'milk red protein' or 'lactotransferrin'. It is the iron-binding protein initial discovered in breast milk, however, it is also found to be in cells such as the leukocytes' neutrophils and bodily secretions such as the saliva, tears, though it should not be muddled up with the 'milk transferrin' in the milk of many animal species like the rabbit.
- **The *ovotransferrin*** is commonly known as 'ovoferrin' or 'conalbumin'. This is the iron-binding protein separated from the egg white of birds.
- **The membrane-bound,** tumour-associated *melanotransferrin*

Primarily, the biological role of siderophilin is in conveying iron through vertebrates' circulatory system, but for conalbumin and lactotransferrin, there is no recognized precise iron transport role yet. Conversely, the two proteins could take part in inhibiting micro-organisms' growth by depriving them of vital metals because these proteins have a very high affinity for iron and other trace metals. Therefore, they act to guard the milk and egg against getting infected.

2.1 Structure of Transferrin

Transferrin is made up of a one polypeptide chain of approximately 700 amino acid residues structured into the C lobes and N lobes; each of them comprises two sites where synchronization iron takes place (Abdallah &El Hage, 2002; Mizutani et al., 2012; Reyes-Lopez et al., 2015). A short helical fragment links the globular lobes together. An apotransferrin or transferrin protein lacking bound iron forms a complex when it interacts with iron. A molecule of transferrin can bind two bicarbonate ions and two iron atoms to its two precise iron binding sites.

The interaction between iron (Fe3+) and the exact sites for iron-binding on the transferrin is aided by the bicarbonate ions (Park et al., 1985). Welch (1992) stated that there are about 42% same amino acids in the N-terminal domain as are also in the C-terminal domain.

2.2 Synthesis of Transferrin

The liver is the primary organ for synthesizing transferrin, then it is carried by the blood plasma after secretion. Other tissues comprising the mammary gland, brain, spleen, testes, kidney, and ovary have been discovered to remarkably express the gene for transferrin (Lambert et al., 2005, Zakin, 1992; Tu et al, 1991). For tissues (that is, non-liver tissues) where there is a blood barrier separation between the cells and transferrin in the plasma, transferrin synthesis might become vital. In an ordinary circumstance, transferrin binds most of the blood plasma iron (Berhan, 2016).

2.3 Transferrin receptor (transferrin R)

Specific receptors function physiologically by binding transferrin on the surface of the cell and ingesting it, and the transferrin R is used to take up iron bound to transferrin by cells (Reyes-Lopez et al., 2015). All nucleated physiological cells express the transferrin R, which aids cells of vertebrates to take up iron through the transferrin endo and exocytosis cycles (Richardson & Ponka, 1997). Liver cells, brain, red blood cells, monocytes, thyroid cells,

intestinal cells, the blood-brain barrier as well as some bacteria and certain insects have been observed to have the transferrin R (Lönnerdal & Iyer, 1995; Schryvers et al, 1998), and with less affinity to apotransferrin compared to the diferric transferrin; various transferrin R are of vastly varying transferrin affinities (Sun et al, 1999). There are two well-known forms of transferrin R, namely, the transferrin R1 and transferrin R2. Of the two receptors, Transferrin 1 is the best characterized and expressed the most. Transferrin R1, with a molecular mass of ~190 000 Dalton, is a glycoprotein.

on the membrane with a homo-dimeric nature binding, in a fashion that is pH-dependent, two transferrin molecules, and permits iron transport into cells (Bou-Abdallah, 2012).

In the head of the common carp's (*Cyprinus carpio*) kidney, Chen *et al*. (2013) observes that there is an interaction between the transferrin R and the zinc-transferrin complex which triggers immature red blood cells multiplication. A high level of iron is needed for malignant cell growth; hence, the levels of the expression transferrin R are high (Huebers & Finch, 1987). In the physiological immune response to infection by bacteria, transferrin R is also described to take part (Ding et al, 2015)

3.0 Uptake of iron from transferrin by cells

A receptor protein of the cell membrane that is transferrin-specific is responsible for taking up transferrin bound iron in the cells. Iron-occupied transferrin and the transferrin R bind on the surface of the cell, and the endosomes confine the complex of Transferrin and Transferrin R through coated vesicles and coated pits. The endosomal membrane ATPase proton-pumping action quickly acidifies, at about pH 5–5.5, the lumen of the vesicle. Iron transport from the transferrin is aided by the endosomal low pH, and the iron is mobilized across into the cytosol from the membrane of the endosome. Transferrin R is tightly bound to the apotransferrin at the optimal endosomal lumen pH.

The complex of transferrin R and apotransferrin escapes breakdown by the lysosome sorting into vesicles of the exocytic. The plasma membrane and the vesicle of the exocytic bind together and expose the complex of transferrin and apotransferrin to the pH outside the cell. Apotransferrin and transferrin R separate so that they can undergo another binding of endocytosis or exocytosis and transferrin cycle as their apotransferrin has a very low affinity have a very low for the receptor (de Jong et al, 1990; Thorstensen & Romslo, 1990).

Virtually all the iron in the serum usually binds to transferrin. Transferrin (diferric transferrin) occupied by iron is bound to the transferrin R on the surface of a cell and via a clathrin-dependent pathway, the complexes become endocytosed. The $Fe3^{+}$ (ferric iron) and transferrin separate while the complex left goes back to the plasma membrane when pH lowers through the maturation of the endosome. Apotransferrin separates from transferrin R to allow for another cycle of taking up iron when the surface of the cell is at a neutral pH (Chen & Paw, 2012). Jabeen *et al*. (2015) studied the efficient flexibility of transferrins from four channids (Genus, *Channa*: Channidae) that breathe air and how it is significant to their continued existence. They concluded that at acidic pH, a remarkably large quantity of iron is maintained by transferrin (Jabeen et al, 2015). In the event of respiratory acidosis, it must be vital, at low pH, for free iron to be secured, as free iron, in form of $Fe3^{+}$, precipitates when the oxygen is low, even at biologically optimal pH.

4.0 Test for transferrin

The standard transferrin range in the laboratory is 204-360 mg/dL. Physiologically, the amount of transferrin can be employed in measuring the level of iron besides other biological indicators. In determining the ability of blood to carry iron, examining the metabolism of iron, and determining anaemic causes, researchers employ the test for the level of Transferrin. To read the level of transferrin saturation, other laboratory tests like TIBC and serum ferritin in addition to the saturation level must be performed it cannot be read alone in seclusion. In

diagnosing anaemia caused by the deficiency of iron, because ferritin is more sensitive than transferrin, it is the first indicator to become low (Waldvogel-Abramowski et al., 2014). The test that assesses the ability of the blood to bind iron with the transferrin is the Transferrin or Total iron-binding capacity (TIBC).

5.0 Chemical properties of Transferrin

- ***Amino acid composition***

Several scholars have made reports on how the amino acid for the physiological transferrin is composed (Putman, 1975). Transferrin expresses a few rare characteristics but for a total lack of available sulphydryl groups, and an increased amount of half-cystine consistent with 19-intrachain disulphide bonds. The transferrins of various species usually possess relatively identical structures of amino acids. Conversely, the slight dissimilarities expressed in the structures of their amino acid alongside the disparity in the contents of their carbohydrate yields variances in their kinesis during electrophoresis. Transferrin displays wide polymorphism in their genes and disparity in their kinesis during electrophoresis typically observed to be caused by substitutions of amino acids, especially where the phenotypes of the gene are from identical species. For instance, research that compared the digests of chymotrypsin from transferrin C and D1 of humans showed that an amino acid residue of aspartate in transferrin C is perhaps substituted by a residue of glycine in transferrin D1 (Wang & Sutton, 1965). Conversely, a research carried out on equestrian transferrin D and R, the study observed that a residue of glutamate and aspartate in transferrin D are substituted by two residues of glycine in transferrin R (Chung & McKenzie, 1983).

- ***Amino acid sequence***

Human transferrin comprises 678 amino acid residues, which in addition to the two moieties of glycan, have a total molecular weight of 79 550 Da. The structure indicates broad homology at the core, with the N-terminal region containing 1-336 residues and the C-

terminal region containing 337-678 residues, having 40% of the residues alike. There is also a similar case in the structure of ovotransferrin (William et al., 1982) and incomplete lactotransferrin structure (Metz-Boutique et al., 1981). Thus, proposing that there has been an evolution from the structural gene of the transferrin molecule, a familial protein having a single site for binding of metal and by a gene duplication process, of nearly 340 residues of the amino acid (MacGillivray et al., 1982). Williams *et al* (1982) suggested that this familial protein is a membrane-bound metal-receptor protein and not a serum protein as the separated ovotransferrin quickly lost half-molecule from the bloodstream through the kidneys. Conversely, Mazurier *et al* (1983) suggested that, in contrast, the transferrins might have a 6-fold homology, and that the two domains with the most homology are sited in the two iron-binding sites of the protein.

- ***Carbohydrate content***

Transferrins are all glycoproteins. Transferrins show more variation in species than in the composition of their amino acids the composition of their carbohydrate is the basis for comparison. Therefore, they are described to have from 1-to 4 carbohydrate chains per molecule and the overall content of their carbohydrate ranges from 3.0 to 11.8% protein weight. About 6% of the protein weight of human siderophilin is a carbohydrate moiety. It is expressed as two similar, branched hetero-saccharide chains or glycans which attach to the asparaginyl residues' amide groups by 13-N-glycosidic linkages. Conversely, reports stated that this alongside, a negligible number of transferrin that possess only hetero-saccharide chains that are tri-branched (Kerckaert & Bayard, 1982). Various researchers have cautiously elucidated these hetero-saccharide chains structure. Findings have described each hetero-saccharide chain to comprise three mannose, two galactose, four N-acetylglucosamine and two sialic acid residues (Dorland et al., 1977). In the chain's terminal region of the chain are located the residues of sialic acid, which are easily prone to neuraminidase excision. A

'biantennary' structure can be used to represent the total structure of carbohydrates in the hetero-saccharide chains as presented in Fig. 1. In the C-terminal domain of the protein, two hetero-saccharide chains are said to be in attachment with asparagine residues 413 and 610, with evidence from the identified human siderophilin amino acid sequence (MacGillivray, 1982).

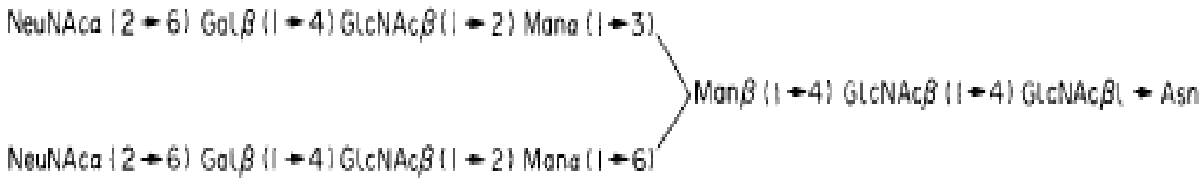

Figure 1. Glycan Structure of Human Serum Transferrin (Chung, 1984).

- **Metal-ion binding of Transferrin**

In the presence of the ions of bicarbonate, Iron*(Ill)* Transferrin binds with two Fe^{3+} ions to yield a pinkish compound that maximally absorbs light at 465-470nm. The pH affects this reaction: it has an optimal pH range of 7.5-10, but at a lower pH, complete dissociation takes place at pH 4.5, while it incompletely dissociates at pH 6.5. Therefore, this nature makes it widely useful in the *in vitro* preparation of apotransferrin.

For all the protein bound Fe^{3+} ions, concurrently, a single bicarbonate ion binds with the release of three protons. Therefore, the complete transferrin and Fe^{3+} ions reaction can be denoted by the equations as shown in fig 2:

$$Fe^{3+} + H_6Tf + HCO_3^- \rightleftharpoons [Fe\text{–}H_3Tf\text{–}HCO_3]^- + 3H^+ \quad (1)$$

$$Fe^{3+} + [Fe\text{–}H_3Tf\text{–}HCO_3]^- + HCO_3^- \rightleftharpoons [Fe_2\text{–}Tf\text{–}(HCO_3)_2]^{2-} + 3H^+ \quad (2)$$

Figure 2. An equation of the reaction between Fe^{3+} and Transferrin (Chung, 1984)

While there is common credence that the three protons given off for each Fe^{3+} ion binding in the reaction are a resultant of the three tyrosine residues of the protein that ionizes (Gelb &

Harris, 1980), however, a study, proposes that perhaps two tyrosines solely take part in the formation of complex, and the third proton that is given off from the Fe^{3+} ion bound molecule of water (Pecoraro et al., 1981). As established by studies on the kinesis during electrophoresis, in this reaction, transferrin bound by two Fe^{3+} ions (differic) receive two net negative charges (Warner & Weber, 1953). Bicarbonate is proposed to be the negative ion (anion) that takes part in the process of binding based on the evidence of this 'charge balance'. Conversely, nuclear magnetic resonance spectroscopy, equilibrium binding and potentiometric titration research associate the anion bound to carbonate (Aisen & Listowsky, 1980).

The intestine absorbs the Fe^{2+} form of iron and it likely proceeds into the circulation as a Fe^{2+} ion. There are suggestions that the protein of the serum, caeruloplasmin (ferroxidase) is responsible for the catalysis of oxidizing of Fe^{2+} to Fe^{3+} ion to enable it to bind to transferrin. Conversely, from a biological perspective, it is important to identify whether transferrin could bind to the Fe^{2+} ion as well. Most of the current evidence proposes that Fe^{2+} ion does not bind to it, and sparingly if it binds at all. However, according to Kojima and Bates (1981), where an oxygen molecule and carbonate ion is present, the Fe^{2+} ion can bind to an apotransferrin to first yield a transitional ternary Fe^{2+}-transferrin-CO~--complex, that further oxidizes in the presence of molecular oxygen to yield a more stable Fe^{3+}-transferrin-CO~--complex.

- ***Other metals***

Apart from Fe^{3+} ion, various divalent, trivalent, and tetravalent metal ions also bind to transferrin. Some of these are transition metals, elements of the actinide and lanthanide series as shown in Table 1 (Chasteen, 1977; Gelb & Harris, 1980; Pecoraro et al., 1981). These metals with transferrin have a similar reaction mechanism to the one for the binding of Fe^{3+} ion. Conversely, in the complete reaction, the total bound metal ions and the number of released protons depend on the radius of the metal ions and tendencies for hydrolysis (Gelb &

Harris, 1980; Pecoraro et al., 1981). For instance, research on the release of protons indicated that when two Al^{3+} ions bind with transferrin it gives off six protons, whereas a related reaction involving Cu^{2+} ion releases just four protons. The rationality behind this is based on that whenever a metal ion binds, an ionization of two residues of co-ordinated tyrosine yields two protons, whereas the metal ion hydrolysed yields the protons left, if at all. Conversely, the metal ions' ionic radii supposedly impact the stoichiometry of the reaction involving the binding of metal.

Though transferrin is relatively well-known to bind with two ions of $A1^{3+}$ and Cu^{2+}, other investigations propose that metal ions having larger ionic radii than europium (0.095 nm) binds with just a single metal-binding site of transferrin. The C-terminal region of transferrin contains this larger site (Pecoraro et al., 1981).

Table 1. Transferrin's Metal Binding Ions of (Pecoraro et al., 1981)**.**

Metal Ion	Ionic Radius (nm)	No. of metals bound	No. of Tyrosine residues
Cu^{2+}	0073	2	4
Zn^{2+}	0.074	2	3.7
Fe^{3+}	0.0645	2	4.2
Eu^{3+}	0.095	2	4.2
Th^{4+}	0.094	2	2.9
Nd^{3+}	0.0983	1	2.2
Pr^{3+}	0.099	1	1.8

5.1 Nature of the iron-binding sites

Understanding the nature of the two iron binding sites of transferrin has been one of the essential areas in its study. Therefore, there has been significant interest in determining if the two protein sites are the same in structure and function, and any disparity exists in the interaction and affinity for binding between the two protein sites when the iron is binding.

In their early research, Warner and Weber (1953) demonstrated that the transferrin-metal binding was very cooperative, and thus involved a binding mode regarded as pairwise.

Conversely, Aasa *et a1*(1963) in the subsequent study demonstrated that there was a nearly equivalent association constant for the two iron atoms' binding. Hence, the conclusion was that there are two equal and independent sites in transferrin and a random binding of the two iron atoms. In contrast, another data submit that the process of binding is sequential, that is, not random or pairwise, and the sites are *non-equivalent:*

$$Tf + Fe \overset{K_1}{\rightleftharpoons} FeTf + Fe \overset{K_2}{\rightleftharpoons} FeTfFe$$

Figure 3. Transferrin binding process with Fe (Iron)Chung, 1984**.**

This deduction is buttressed by the chemical and spectroscopic data as follows:

(i) From an EPR spectroscopy two sites were shown to be distinct when Chromium ion, Cr^{3+} and vanadyl ion, VO^{2+} occupies them(Aisen & Listowsky, 1980; Chasteen, 1977). The variance in charges of the ligand groups at the two sites may be the reason for this difference in spectroscopy.

(ii) Dependency of the binding of Iron on pH: when the chelating agents are lacking, the C-terminal region will still be occupied till as low as pH 4.8 while at any lower than pH 5.7, the N-terminal region would not bind with iron (Princiotto & Zapolski, 1975). Also, at pH 7.4, the stoichiometric binding constant of the N-terminal region is 5 times lower than the C-terminal region, and at pH 6.7, it is raised to a factor of 33 (Aisen & Listowsky, 1980). Conversely, as their iron affinities are not considerably altered with or without the other being occupied, hence, the regions (C-terminal and N-terminal) are nearly independent.

(iii) At the point when pH is neutral, there is a specificity of the chelate where at low pH, iron is directed to the C-terminal region by $Fe^{III}(nitrilotriacetate)_2$ while the iron is directed to the N-terminal region by $Fe^{III}(citrate)_4$ (Aisen & Listowsky, 1980).

(iv) The two sites are not occupied similarly in fresh serum of humans: the N-terminal region is preferably occupied, and it becomes even more preferred when incubated at 37°C. Conversely, the C-terminal region becomes more preferred when the serum is stored at - 15°C (Williams & Moreton, 1980).

5.2 Structure of metal-binding sites

There is a better understanding of the transferrin metal-binding sites' structure as revealed by several studies. For instance, there is a report that the transferrin metal-binding sites are positioned below 1.7 nm under the protein surface (Yeh & Meares, 1980; Zweier et al., 1981).

Furthermore, according to the outcomes from several physicochemical procedures, there is now a well-known conclusion that the ligands of transferrin iron binding-site include an ion of bicarbonate or carbonate, a hydroxide ion (from water), two histidines, and two tyrosines that combine with Fe^{3+} ion to become a six-coordinate complex (Pecoraro et al., 1981).

There is a paper on the whole siderophilin sequence (MacGillivray, 1982) and conalbumin (Williams et al., 1982). Chasteen (1983) suggested that the bicarbonate ions and Fe3+binding sites are perhaps positioned close to the two peptide fragments intersection linked in the human transferrin N-terminal region by Cys-117 to Cys-194. The likely metal ligands are two among three histidines, 119, 207, and 249, and the two tyrosine, Tyr-185 and Tyr-188, while the carbonate anion binding site electrostatically binds with Arg-124, and/or the Lys-ll5, Lys-ll6, His-ll9 sequence. The amino acids are sequenced identically as in the C-terminal region (Chasteen, 1983). Settling the role of these residues of amino acids requires observing the protein with high-resolution X-ray crystallography.

5.3 Negative Ion by Transferrin

- ***Anions***

In biological systems, it is well-known that for the specific transferrin-Fe^{3+} ions binding to occur, a carbonate or bicarbonate anion must also bind. The word 'synergistic' is used to define this process of anion binding because of the binding metal ion completely needing anion. Other negatively charged ions like thioglycolate, pyruvate, nitrilotriacetate (NTA), Phenylalanine, oxalate, glycine and so on, will aid the binding of iron when carbonate or bicarbonate are lacking (Schlabach and Bates, 1975). It is noteworthy that there is a carboxyl group and second-electron withdrawing group, usually, a second sulphdryl, carboxyl or an amino group, positioned not farther than 0.63nm from the initial carboxyl group, which are capable of imitating a structure like a carbonate. A common formula as shown in fig. 4 can be used to represent these negative ions, where the 'L' depicts the closely located electron-withdrawing functional group. Conversely, transferrin binds most tightly to the carbonate or bicarbonate anions, which when present in the medium acts on the ternary complex of Fe_2^{3+}-transferrin-anion to displace other anions.

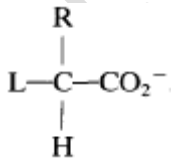

Figure 4. Formula representing negative ions that bind with transferrin

6.0 Biological functions of transferrin

6.1. Transferrin as a transporter of iron and metal ions

Physiologically, transferrin regulates the amount of free iron. It binds, isolates and conveys Fe^{3+} ions to inhibit masses of insoluble ferric hydroxide from being deposited and conserve the iron available. Transferrin functions primarily to mobilize iron from the reticuloendothelial cells, liver and intestine to tissues lacking iron for usual development and growth. Transferrin also takes part in immunity mostly through its iron (Fe^{3+}) binding ability (Herath et al., 2015; Bai et al., 2016). Sun *et al*. (1999) suggest that transferrin perhaps takes part in the mobilization of several metal ions apart from iron, examples are certain metal ions

that are toxic, metal ions for therapy and metal ions for radiodiagnosis. When there is a low concentration of serum albumin, only about 30% of other metals can bind to transferrin without needing to displace the iron that binds more tightly, because iron occupies the sites for binding of metals on transferrin. From postulations, transferrin has substantially taken part in mobilizing Ru^{3+}, VO^{2+} (V^{4+}), Bi^{3+}, Ti^{4+} and Cr^{3+}, all-metal ions that are probably relevant in therapy. As the trivalent ion, the mobilization of manganese may involve the action of transferrin.

Conversely, transferrin is likely also involved in the transport of actinide ions, comprising Pu^{4+} and Al^{3+}, to the tissues (Vincent & Love, 2012). According to De Smet *et al*. (2001) common carp's transferrin has been accepted as the key protein for mobilizing non-iron metals, like cadmium (De Smet et al., 2001). In Nile tilapia [*Oreochromis niloticus* (*O. niloticus*)], the levels of serum transferrin increased due to times of cadmium or zinc exposure, similarly proposing that transferrin is used as a factor in biologically detecting the toxicity of heavy metals in fish (Firat & Kargin, 2010). Dietrich *et al*.(2011) stated that transferrin from seminal plasma of carp can preserve the motility of sperm from the toxicity of cadmium when they indicated the tendency of cadmium ions to bind the protein-transferrin in seminal plasma of major carp and to defuse the cadmium toxicity on the motility of carp sperm (Dietrich et al., 2011).

6.2. Transferrin as antimicrobial agent

Transferrin can be an antimicrobial agent because of its high affinity for binding iron binding. Its capability to lower the level of free iron in the serum, thereby creating an environment low in iron which restricts microorganism pathogens from causing an infection is what makes it play a role in immunity (Suzumoto et al, 1977; Chen et al., 1999).

Soluble elements that prevent the growth of microorganisms facilitate the humoral intrinsic immunity (Aoki et al., 2008). Conversely, transferrin acts adversely at the severe stage in

cases of inflammation (Bayne & Gerwich, 2001). Conalbumin and lactotransferrin possibly have antimicrobial activity as well, which must be in direct contact with the bacteria instead of simply depriving it of iron (Damastri et al, 1988). Transferrin has several functions as a protein but it primarily functions to metabolise iron which brings about its function in the intrinsic immune response. The obvious relationship between the mechanism for immune response and transferrin suggests the protein is a potential disease-resistant gene (Gracia-Frenandez et al., 2011). According to Liu *et al*. (2010), there was a considerable increase in expressed transferrin control in catfish (*Ictalurus punctatus*) after it was infected with *Edwardsiella ictaluri*, the disease pathogen of enteric septicaemia (Liu et al, 2010).

In their studies, Kovacevic *et al*. (2015) used quantitative PCR to observe the genes expressed that code for the severe stage proteins throughout an infection by *Trypanosoma carassii* in the goldfish (*Carassius auratus* L.) and found that there was an increase in the transferrin control during the progression of the severe kidney and liver infection, and in the prolonged stage of the infection (Kovacevic et al., 2015). Similarly, Poochai *et al*. (2014) in an experiment, infected tilapia with *Streptococcus agalactiae* and it was observed that iron was deficient in serum of tilapia that was infected with bacteria and a substantial increase in the regulation of expressed transferrin in the fish was discovered which shows the role transferrin plays in intrinsic immune response (Poochai et al., 2014). The levels of expressed transferrin were discovered to increase after rainbow trout was infected by bacteria (Bayne & Gerwich, 2001). Also, the expression of the transferrin gene was found in to increase the spleen and blood's white blood cells (leukocytes) of cod after it was injected intra-peritoneally with bacteria that had been killed by heat (Caipang et al., 2008, Caipang et al., 2009). In the Chinese black sleeper (*Bostrichthys Sinensis*), following a *Vibrio harveyi* infection, there was an observed increase in expressed transferrin gene in the serum

of the Chinese black sleeper primarily in the stomach and liver, serving as a positive acute protein which proposes that serum transferrin takes part in immunity (Gao et al., 2013).

There was an increase of expressed transferrin in the orange-spotted grouper's gill in the course of a *Cryptocaryon irritans* exposure proposing that the host will mostly use the transferrin expressed to make more NO response that significantly functions in the resistance of the host against infection by a parasite (Li et al., 2011). After the initiation of the severe stage, an increased control was confirmed, following transferrin expressed constitutively in the spleen and head kidney [48]. In an investigation by Ercan *et al*. (2013) the transferrin gene of sea bass (*Dicentrarchus labrax*) was observed to be expressed during an experiment where it was infected with *Vibrio anguillarum* and they also described the expressed transferrin gene to increase in the initial 2 days (Ercan et al., 2013). There was also a description of increased control of the expressed transferrin gene after infection by bacteria in sea bass and channel catfish (Neves et al., 2009; Peatman et al., 2007). In goldfish (*Carassius auratus*) and salmonids, particular sites on the transferrin (protein) with relevant functions appeared to experience progressive natural assortment, which indicates a likely connection between fish pathogens resistance and transferrin (Ford, 2001; Yang & Gui, 2004).

6.3. Transferrin as macrophages activator

Located in almost all tissues of animals and across all vertebrate species are the macrophages, and they function importantly in homeostasis and the protection of the host. Being cells found in almost all tissues, macrophages help to keep environments homeostatic, and when there is an infection, they are usually one of the principal kinds of cells to contact pathogens that invade, which is followed by a suitable immune response (Hodgkinson, et al.,2015). In fishes, transferrin functions as the main fish macrophages activator. Products of transferrin cleavage trigger macrophages of fish, though it perhaps characterizes a simple NO initiation pathway in lower vertebrates, it is extremely conserved (Stafford et al., 2001). suggested that products

of transferrin cleavage in goldfish may function as a factor for triggering macrophage by macrophages excitation to yield great volumes of NO. There are several other physiological roles that transferrin play, like differentiation, mobilization of electron and oxygen, growth, processes of cell defence, and synthesis of DNA (de Jong et al., 1990; Welch, 1992; Stafford and Belosevic, 2003; Gomme et al., 2005; Ong et al., 2006; Sun et al., 2012). Additionally, transferrin has been discovered to function as a regulator of hepcidin (a peptide hormone derived from the liver which systemically regulates the movement of iron) upstream (Gkouvatsos et al., 2012).

7.0 Clinical Significance of Transferrin

Of all the nutritional deficiencies worldwide, the deficit of iron deficiency is well-known as the most predominant. Physiologically, the blood transferrin level shows the iron quantity in the body. When transferrin is high, this implies that iron is low, that is, transferrin is bound to less iron, enabling the non-bound iron transferrin to circulate more in the body, indicating that there is likely iron deficiency anaemia. By a way of homeostasis, the liver produces more transferrin so that transferrin binds to iron and mobilizes it to the cells. In the anaemia caused by iron deficiency, receptors of transferrin are Up-regulated (Bermejo & García-López, 2009). Concerning the transferrin-iron complex ratio, low levels of iron in the body are shown by low levels of transferrin that's bound with iron, which has impacts on erythropoiesis and haemoglobin. Clinically, transferrin can be employed in observing erythropoiesis and can identify a deficiency of iron, making it very important.

7.1 Causes of transferrin deficiency

Low levels of transferrin are caused by infection, impairment to the liver resulting in decreased transferrin synthesis, malignancy, Kidney injury or damage causing urinary transferrin loss. In addition, atransferrinemia which occurs when transferrin is lacking due to

a genetic mutation result in liver and heart hemosiderosis eventually causing failure of the liver and heart. Plasma infusion is used to treat atransferrinemia.

When there is an overload of iron, the transferrin in plasma is observed below, that is, iron vastly saturates the transferrin binding site. An overload of Iron could indicate hemochromatosis, which will result in iron deposits on tissues.

Transferrin and its receptors are also related to tumour cells shrinking when the transferrin receptor is employed in attracting antibodies. Elevated saturation of transferrin amplifies the risk of death in cardiovascular patients if their levels of low-density lipoprotein (LDL) and saturation of transferrin are high (>55%) (Wells et al., 2004)

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