Chapter 2

REVIEW OF LITERATURE

2.1. GLYCOPROTEINS

Human plasma is rich in glycoproteins, many of which exist in different glycoforms. Several reports have been published on altered glycosylation of plasma proteins in different pathological conditions [78, 79]. Aberrant glycosylation of cell surface glycoconjugates and serum glycoproteins are involved in inflammative cell differentiation, infection, tumour progression and metastasis. Changes in protein glycosylation are early indications of cellular changes in many such diseases and provide useful diagnostic markers and insights into disease progression and pathogenesis [80].

Sarcicone et al. in 1967 [81] isolated a highly heterogeneous glycoprotein, the 1-Acid Glycoprotein (AGP) from human liver. It has a molecular weight of 41-43 kDa when examined under SDS-PAGE and contained 45% carbohydrates [82]. It was identified as the complex type of 5 N-linked glycans. Significant heterogenicity of the oligosaccharides existed due to N-acetyl neuraminic acid (NeuAc) linked to galactose (Gal) and due to the presence of Fucose (Fuc) residues [83]. Marked changes in the glycoforms of human AGP were observed in acute-phase reactions with alterations in the branching pattern [84]. Nakano et al. [85] with the help of HPLC and MALDI-TOFMS analyzed carbohydrate chains of human, bovine, sheep and rat 1- Acid Glycoprotein and found that these chains showed distinct variations in different animals. The glycosylation of AGP has been
studied in patients with estrogen treatment, acute and chronic inflammation and malignant diseases [78, 86-89]. Branching of the attached oligosaccharides was found to be decreased in acute inflammatory conditions while it was increased in chronic inflammation like RA [90].

2.1.1. Organ and Species specific N-glycosylation

Comparative study of the sugar pattern of the same glycoproteins produced in the same organ of different animals and those produced in different organs of the same animal revealed organ and species specificity in N-glycosylation [91]. The major sugar chains of $\gamma$-Glutamyl transpeptidases ($\gamma$-GTPs), a membrane-integrated glycoprotein of epithelial cells form an example. Various organs in all mammals showed this phenomenon. It was observed that bisecting GlcNAc is detected in the sugar chain of kidney enzymes from man, mouse, rat and cattle. But the bisecting sugar chains were absent in liver enzymes of all these animals. None of the liver glycoproteins contain bisected sugar chain indicating that expression of Gn-T III is suppressed by hepatocyte differentiation. Enzyme is strongly expressed in the kidney cells of all mammals [91].

Site directed N-glycosylation was established by comparing the oligosaccharide patterns of the two subunits of human Chorionic Gonadotropin (hCG) [92]. This glycoprotein hormone produced by trophoblasts of placenta is a heterodimer composed of $\alpha$ and $\beta$ subunits. Altogether five N-linked sugar chains have been identified of which two are seen on each subunit. Molar ratio of oligosaccharides in the two subunits suggested that these four potential N-glycosylation sites of hCG gave different ratios of each oligosaccharide indicating the presence of strict site directed N-glycosylation. High levels of
hCG were detected in the blood and urine of patients with a variety of trophoblastic diseases as well as pregnant women. Urinary and serum hCG levels can be used as a marker for the diagnosis and prognosis of trophoblastic diseases and normal pregnancy. Also, the altered N-glycosylation of hCG in malignant cells was found to produce abnormal sugar chains which could not be detected in normal hCG [93].

According to ‘Warren-Glick’ phenomenon proposed by Robbin’s group [91] the expression of large N-linked sugar chains on the surface of malignant cells should be considered as the first evidence to have indicated the malignant alteration of N-glycosylation. It was proposed that the molecular basis of ‘Warren-Glick’ phenomenon is the increase of tri- and tetra- antennary complex type N-linked sugar chains in malignant cells.

Kobata [91] suggested that some of the altered glycosylation of proteins produced by tumour cells are species specific. But ectopic expression of bisected sugar chains in γ-GTP purified from hepatoma of rat and its absence in human hepatoma offered a good evidence for this conclusion. The γ-GTP purified from human hepatoma was found to be more enriched in the tri- and tetra- antennary sugar chains indicating the expression of ‘Warren-Glick’ phenomenon in this glycoprotein. Abnormal biantennary sugar chains found in the Choriocarcinoma hCG was also observed in this γ-GTP.

Pure carbohydrates were found to be incapable of major histocompatibility complex (MHC) binding and T-cell simulation. But T cells can recognize glycoproteins carrying mono- and disaccharides provided their glycans are attached to the peptide at suitable positions. This T cell recognition of glycopeptides may be important in immune defense against microorganisms, because many microbial antigens are in fact glycosylated. T cell recognition of glycans may play an
important role in the immune defense against tumours because cancer cells show abnormal glycosylation of proteins in malignant cells [94].

### 2.1.2. Glycosylation

Rudd et al. [95] has reviewed glycosylation with reference to immune system. The biodiversity in protein glycosylation plays an important role in the biosynthesis and biological activity of the glycoproteins involved in antigen recognition. During the transport of the glycoprotein at the time of secretion, sugar chains undergo successive modifications by the glycosylation processing enzymes. This process ensures the relevant glycan structure suitable or its function. Also, the sugars play a role in protein folding and assembly, quality control, ER associated retrograde transport of misfolded proteins and generates, loads and influences the antigenic peptides into MHC class I and II. By virtue of their size, glycans shield large region of protein surfaces protecting the immune molecules from proteolysis. The events involved in these processes have been explained in detail [95].

Glycosylation differences between normal and pathogenic prion protein isoforms were elucidated by Rudd et al. [96]. Prion protein consists of an ensemble of glycosylated variants or glycoforms. Each contains two conserved N-glycosylation sites (Asn-181 and Asn-197 in Syrian hamster). The functions of sugars have not yet been established. Alterations in the populations of sugars attached to these proteins reflects changes caused in disease. Transmission of prion diseases, also called Spongiform encephalopathies, involves conversion of normal prion protein (PrP<sup>c</sup>) into a disease causing conformer (PrP<sup>Sc</sup>). It was revealed that though the normal (PrP<sup>c</sup>) and pathogenic (PrP<sup>Sc</sup>) prion proteins of Syrian hamster contained the same set of 52 bi-, tri- and tetra antennary
N-linked oligosaccharides, the relative proportions of individual glycan differed among the two categories. Also, PrP\(^c\) contained decreased levels of glycans with bisecting GlcNAc residues and increased levels of tri- and tetra antennary sugars. This change is consistent with a decrease in the activity of N-acetyl glucosaminyltransferase III (Gn-T III) in cells where PrP\(^{Sc}\) is formed. It was also reported that in PrP\(^{Sc}\) there are no glycans associated with the disease state that are not present in the normal case unlike in RA where the presence of agalactosyl IgG correlated with the disease activity. An alteration in Gn-T III activity noted in RA had resulted in an increase in oligosaccharides containing bisecting GlcNAc [96].

Jikko et al. [97] reported the effects of X-irradiation on metabolism of proteoglycans and Type II collagen in a rabbit chondrocyte culture system. Irradiation of an immature culture in which chondrocytes had just reached confluence, suppressed incorporation of sulphate into glycosaminoglycan. On the contrary, irradiation of a mature culture in which the chondrocytes had already secreted extensive cartilage matrix, did not affect the rate of synthesis of proteoglycan (incorporation of sulphate), but stimulated the degradation of proteoglycans. The synthesis of Type II collagen was not affected in either of the cases. It is obvious that metabolism of proteoglycans is more radiosensitive than that of collagen, the reason of which is yet unknown. Cornelissen et al. studied a dose and time dependent radiation inhibition of glucosamine incorporation into the tibia cartilage in chick organ culture [98]. They suggested that the initial stage of apoptosis which is the appearance of acid phosphatase in vacuoles, cytoplasm and surrounding extracellular matrix of the irradiated cartilage was involved in the inhibition.
The sugar chain structure of some glycoproteins could be modified by aging as revealed by a decrease in the galactose content of IgG in aged persons [91]. It was reported that the galactose deficiency decreases the complement binding potential of IgG. This explained the phenomenon of immunodeficiency observed in aged persons.

Dementia is one of the most important targets of aging research because it severely lowers the quality of life in an aged person. β–amyloid, a glycoprotein was found to be deposited in the brains of patients with Alzheimer’s disease. Studies revealed that a protein called Amyloid Precursor Protein (APP) is a precursor of β–amyloid. A large amount of APP is produced in healthy brain. A mechanism to induce an abnormal cleavage of APP is considered as a key step in this line of study. As APP is a glycoprotein, study of its sugar chains and age related alterations of their structures could be significant [91]. This may solve various pathological problems found in elderly people. Functional aspects of the sugar chains of glycoproteins in brain and nervous system is being investigated.

Aberrant glycosylation of glycoproteins that are either presented on the surface or secreted by cancer cells is a potential source of disease biomarkers and provides insights into disease pathogenesis. Hamid et al. [99] sequenced the N-Glycans of the total serum glycoproteins from advanced breast cancer patients and healthy individuals by HPLC coupled with exoglycosidase digestions and mass spectrometry. They observed a significant increase in a trisialylated triantennary glycan containing α1, 3-linked fucose which formed part of the sialyl Lewis^x^ epitope. These preliminary findings suggested that specific glycans and glycoforms of proteins may be candidates for improved markers in the monitoring of breast cancer progression [99].
Harvey et al. [100] had reported that the level of Chondrex, a major secretory glycolprotein in human chondrocytes and synovial fibroblasts is increased in serum of patients with joint and cartilage diseases. They assigned the normal values of Chondrex as 25 – 95 µg /L for healthy adults. Values for patients with RA or OA were significantly greater than in normal healthy adults, inactive RA patients and diabetic patients. During antirheumatic drug therapy, Chondrex values decreased in responders and remained unchanged in non-responders. Chondrex was suggested to be a useful marker in the clinical investigation of arthritis [100].

2.1.3. Glycosylation changes of IgG in Rheumatoid Arthritis

The integral feature of γ-immunoglobulin (IgG) antibody is the conserved N-glycosylation site on the Fc at Asn 297. N-linked oligosaccharides may be attached in the Fab region too, the frequency and location depending on the occurrence of Asn-Xyl–Ser/Thr sites. The average number of oligosaccharide chains per IgG molecule is 2.5: 2 in then Fc region and the remainder in the Fab. Each sugar chain has either two terminal galactose residues (G2), one galactose and one N-acetyl glucosamine (G1) or two terminal N-acetyl glucosamine and no galactose (G0).

The auto antigenic reactivity has been localized to the constant region (C2 domains) of IgG. There was no evidence for a polypeptide determinant, but carbohydrate changes have been reported. Parekh et al. [71] compared the N-glycosylation pattern of serum IgG isolated from normal individuals and from patients with RA and primary Osteoarthritis. The results showed that IgG of normal individuals and the patients contained different distributions of asparagine-linked, bi antennary complex-type glycans. No new oligosaccharide
structures were detected. The relative extent of galactosylation was compared with that of normal individuals. One or both the arms of IgG molecules from patients terminated in N-acetyl glucosamine revealing a failure in galactosylation. These two were considered as glycosylation diseases which reflected changes in the intracellular processing or post-secretory degradation of N-linked oligosaccharides.

A good amount of research has been done on agalactosylation of IgG in inflammatory diseases like Rheumatoid Arthritis [71-103], Osteoarthritis [71], Spondyloarthritis [100] etc. Parekh et al. (1985) reported that the oligosaccharides in the IgG from RA patients were deficient in galactose and contained increased levels of G0 glycoforms [71]. These findings have been confirmed and extended in 1989 by the same researchers [104]. G0 levels correlate with the disease severity in patients with RA and revert to normal in Rheumatoid Arthritis sufferers who are in pregnancy induced remission [102]. Malhotra et al. compared the glycosylation of the Fab and Fc fragments from IgG of normal and RA patients and stated that the alteration in galactosylation is restricted to Fc region only [101]. This alteration in IgG galactosylation in RA creates a new mode for the interaction with complement by binding to the collagenous lectin, the Mannose-Binding Protein (MBP). MBP binds with terminal Fucose, glucose, mannose or glucosamine residues with its carbohydrate recognition domains (CRDs), but not with galactose. By NMR studies it was showed that terminal sugar (glucosamine) became exposed and was accessible to MBP only in those molecules in which Fc oligosaccharides lacked galactose. They have also demonstrated that multiple presentation of IgG-G0 glycoforms to MBP resulted in activation of the Complement leading to chronic inflammation of synovial membrane in the affected joint.
Through a series of research in Dwek’s laboratory (Glycobiology Institute, University of Oxford, England) it was shown that increased expression of agalactosyl IgG glycoforms occurs in patients with adult RA [71], in Crohn’s disease and in a form of leprosy called erythema nodosum leprosum [103]. It is altered in the juvenile onset of RA, mature RA patients and in tuberculosis [104]. Another study in which agalactosyl IgG was injected into mice showed that this glycoform was pathogenic and induced arthritis [105]. Elevation of agalactosyl IgG was noticed in patients with active Spondyloarthropathy [106]. The level of agalactosyl IgG was reported to be high in Takayasu’s arteritis [107] and in Systemic Lupus Erythrematosus [108]. Tomana et al. analyzed IgG from 30 SLE patients using Gas Chromatography and observed an abnormal glycosylation in 60% of the patients [109]. In SLE patients, presence of agalactosyl IgG antibodies during pregnancy seemed to be pathogenic for the foetus. Maternal – fetal transmission of these antibodies has been associated with congenital heart block [110].

The mechanism of agalactosylation of IgG is not completely understood. Axford et al. (1987) had reported reduced galactosyl transferase activity of B cells in RA patients reflecting the inefficiency in IgG galactosylation [111]. Cytokines and oxygen radicals have been suggested to contribute to the agalactosylation [105]. An increase in IgG galactosylation was observed in RA patients after treatment with Methotrexate (MTX) alone or in combination with Remicade, an anti TNF-α [112]. Changes in the glycosylation of IgG in collagen induced arthritis (CIA) was studied by Jones et al. [113] who reported less galactose on the IgG of arthritic mice. N-acetyl glucosamine content of IgG
was significantly elevated. There was no difference in sialic acid content in normal and arthritic mice. The similarity in glycosylation changes in CIA and in patients with RA suggests that a common pathogenic mechanism may be involved. Gornik et al. [114] suggested that the decreased galactosylation of IgG in RA precedes the outbreak of the disease creating novel, potentially immunogenic structures and lead to other symptoms. In their opinion, agalactosylation of IgG thus might be one of the causes and not the consequences of the disease.

In RA patients, the IgG is increasingly agalactosylated exposing the N-acetylglucosamine residue as the terminal sugar. The rheumatoid factor (RF), an antibody (a form of IgM) formed against the IgG0 molecules increases in RA and is therefore used for the diagnosis of the disease [115].

Also, the determination of agalacto-IgG may aid in the diagnosis and treatment of RA. The decrease in galactosylation of IgG leads to an increase in terminal N-acetylglucosamine residues. IgG from RA patients and IgG from control individuals were discriminated with respect to its content of terminal N-acetylglucosamine recently by Liljeblad et al. using affinity chromatography [116].

Loss of proteoglycans from articular cartilage was reported by Gillard and Lowther in carrageenin induced arthritis [117]. They assessed 20% loss of proteoglycans within 24 hours and a further 30 - 60% loss within 5–7 days. The chondrocytes replaced the lost proteoglycans within 42 days. They had also estimated 40 - 75% decrease in the rate of proteoglycan synthesis by inflamed articular cartilage in vitro and in vivo. However, chondrocytes recovered the
synthetic ability and showed a rate of synthesis above those of controls. Thus there was a replacement of proteoglycan lost during the initial period of inflammation.

Exer and co-workers [118] reported an increase in metabolic turnover of proteoglycans in the cartilage and ligament during chronic inflammation in adjuvant arthritis. Trnavska et al. [119] demonstrated that alteration in the metabolic turnover of collagen manifested by an increase in the break down of newly formed collagen in acute phase and by retardation in the conversion of soluble to insoluble collagen in chronic phase of arthritis. Huckerby and colleagues characterized oligosaccharides of chondroitin sulphate under NMR using radio-labeling [120]. Their data helps to alleviate some of the difficulties resulting from signal complexity.

The molecular pathogenic mechanism of RA remains to be further defined and is still a great challenge in determining the diagnosis and in choosing the appropriate therapy in early patients. A study was performed to screen these RA-associated serum proteins by comparative proteomics to provide research clues to early diagnosis and treatment of RA. The protein expression profiles between the two groups were then compared by two-dimensional gel electrophoresis (2-DE) and the proteins over/under-expressed by more than 3-fold were identified by mass spectrometry analysis. Eight proteins which over/under-expressed in the sera of RA patients were identified. In order to define the significance of these candidate biomarkers, further investigations must be carried out to test whether these proteins can be used as useful diagnostic indicators or therapeutic targets of RA [121].
2.1.4. Carbohydrate components

Carbohydrate moieties of glycoproteins may act as ligands in biological recognition in cell-cell interaction, cell migration and proliferation [122-124]. The regulation of leukocyte trafficking relies on the specific interaction between membrane bound lectins, selectins and the oligosaccharidic structure Sialic acid – α (2-3) galactose – β (1-4) Fucose – α (1-3) N-acetylglucosamine (the sialyl Lewis X) that allows leukocytes to roll along the blood vessels. This process is activated and inflammatory cells rush to the site of injury during inflammation [124]. Alterations in glycosylation of cell surface and/or soluble glycoconjugates were reported in many diseases [125, 126]. This is evident in cancer where these alterations disturb the cell adhesiveness leading to metastasis [127-130]. Alternatively, carbohydrates like Sialic acid are essential in self/nonself discrimination by immune system [131]. Afroun et al. [132] had suggested that carbohydrates might be important in macrophage biology. They reported altered glycosylation patterns in murine macrophages upon activation.

2.1.4.1. Hexoses

Reddy and Dhar [133] studied the carbohydrate components of glycoproteins in bone, cartilage, skin, liver, kidney and spleen during acute and chronic phases of adjuvant induced arthritis in rats. They observed significant increase in the contents of total hexoses, hexosamine, Fucose and Sialic acid in all the tissues both in acute and chronic phases of inflammation. The content of total neutral sugar was found to be increased in both acute and chronic phases in all the tissues. The neutral monosaccharides (glucose, galactose, mannose and xylose) were also increased in both the phases of inflammation in all the tissues. However, no significant changes were observed in glucose content of skin and spleen, mannose content of liver and
kidney and xylose content of bone and cartilage during the acute phase of the disease. In addition, the urinary excretions of hexosamine and uronic acid were found to be elevated in arthritic rats. All these explained an increased glycoprotein metabolism in the tissues studied.

Adopting HPAE analysis, Raghav et al. [134] analyzed the monosaccharides in two fractions of proteins (32 kDa and 62 kDa) separated by SDS-PAGE of Concanavalin A –bound plasma protein of RA patients. Only in the fraction 62 kDa, the amount of all monosaccharides was more in the control samples as compared to RA patients. Most of the monosaccharides including N-acetyl glucosamine, galactose and other unidentified ones were found to be proportionately similar in both control and RA samples. But the ratio of glucose / mannose was reversed in controls and RA patients while mannose was increased significantly in RA patients. The high mannose / glucose ratio in RA patients was explained due to the presence of unprocessed oligomannose glycan structure as in the case of certain congenital disorders of glycosylation. Aberrant pentamannosyl hybrid type of glycans were reported to be accumulated in serum glycoproteins due to a defective α-mannosidase II / III activity in congenital disorders [135]. The defect was supposed to be due to the inability to cleave two terminal mannose residues with α 1-3 and α 1-6 linkages to the α 1-6 linked antenna of the core structure leading to the formation of pentamannosyl hybrid type glycans with varying length of α 1-3 linked antenna. The monosaccharides of the 32kDa protein, however, did not show any difference in proportionate sugar profile.
2.1.4.2. Fucoses

Fucose, the 5 methyl pentose is detected in glycans of glycoconjugates isolated from blood group substance, human 1-AGP, human milk etc. It may be external in both N- and O- linked glycoproteins or internal linked to the GlcNAc residue attached to Asn in N-linked species. This can also occur internally attached to the –OH of serine (eg: in tPA and certain clotting factors) [7].

Fucosylated oligosaccharides were reported to constitute major element in immune system of human milk [136]. The oligosaccharides formed up to 12g/L was identified as the third longest constituent of human milk. Human milk oligosaccharides contained a lactose moiety at the reducing end and a Fucose at the non reducing end. There are two types of oligosaccharides based on linkage of Fucose: i) Type I structure with a fucosyl α 1-3 linkage to N-acetyl glucosamine and ii) Type II structure having fucosyl α 1-3 linkage to N-acetyl glucosamine or glucose. Both these types contained another residue, the fucosyl α 1-2 linked to galactose. The addition of fucose to an oligosaccharide by α 1-2 linkage is catalyzed by a fucosyl transferase produced by the secretor gene (Se FUT 2) and that by an α 1-3 or α 1-4 linkage by fucosyl transferase produced by the Lewis gene (Le FUT 3) or other α 1-3 transferase genes (FUT 4, 5, 6, 7, and 9) in this family [134]. Variations in the activities of the FUT 2, FUT 3 or FUT 4 fucosyl transferases are resulted from genetic variations in the concerned genes [137]. Such variations produce milk phenotypes which differ in relative quantities of specific fucosyl oligosaccharides. Women have been classified into non secretors (who do not express measurable 2-linked fucosyl oligosaccharides in their milk or other body fluids) and secretors (who express 2-linked fucoses). Different workers have studied women population around the world based on this
pattern [138,139]. A few studies have also revealed that the expression of milk fucosyl oligosaccharides varies even among secretors [138, 140, 141]. The different blood groups in man are associated with heterogeneous expression of glycoproteins in erythrocytes and other tissues. Genetic polymorphism of the secretor and Lewis genes control the Lewis blood group type [138, 139].

Prevalence of a number of infectious diseases in association with varying expressions of these glycoconjugates have been reported by Blackwell et al. [142]. Differential expression of Lewis or ABO blood group can be associated with infection and diseases of gastrointestinal tract [143, 144]. Neuburg et al., 2004, hypothesized that maternal genetic polymorphism expressed as Lewis blood group types are expressed in milk as varied fucosyl oligosaccharide ratios and also that the milk with higher 2-linked to non 2-linked fucosyl oligosaccharide ratios afforded greater protection against infant diarrhea. Specific fucosyl oligosaccharides thus constitute a major element of an innate immune system of human milk [136].

Variations in the expression of 2-linked fucosyl oligosaccharides in human milk is significantly associated with variation in the risk of disease in breastfed infants, supporting the conclusion that these fucosylated sugars are a fundamental and potent mechanism of protection by human milk against infectious diseases. The active moieties of these protective oligosaccharides may provide a basis for designing novel therapeutic agents for the prophylaxis and treatment of disease.

Blood group types also have been associated with susceptibility to specific diseases, especially of mucosal surfaces. For example, individuals with O blood group are more prone to cholera [143] and certain strains of nor viruses [144] and P blood type more susceptible to haemolytic uremic syndrome [136]. The glycans of
secreted glycoconjugates by virtue of their Fucose moieties can bind to pathogens, inhibit binding by pathogens to their host and thereby protect the host. Secretors whose secretions contain α1-2 linked fucosyl oligosaccharides appear to be less susceptible to urinary tract infections and infection by Candida albicans and Haemophilus influenzae than non secretors [142].

Several reports have been published on glycosylation of plasma proteins in different pathological conditions [78]. Carbohydrate deficient transferrin that lacks one or two complete N-linked complex type oligosaccharide side chains is used to detect and monitor chronic alcohol abuse [145]. α1 Acid glycoprotein (1-AGP), a heavily glycosylated plasma protein with five N-linked complex type oligosaccharide side chains (N glycan) has been studied in patients with estrogen treatment, acute and chronic inflammation and malignant diseases. Changes in branching, sialylation and fucosylation of its oligosaccharides were found [78, 86, 87, 146, 147]. Increased AGP fucosylation was reported in ascetic fluid from a patient with liver cirrhosis [145]. Haptoglobin fucosylation was increased in alcoholic liver disease [146]. Serum cholinesterase fucosylation was increased in liver cirrhosis [147], but not in viral or chronic hepatitis [148]. Increased fucosylation of α-fetoprotein and other serum glycoproteins was found mainly in hepatocellular carcinoma induced by alcohol [149, 150].

Ryden et al. [151] in 2002 studied the diagnostic accuracy of α1 Acid glycoprotein fucosylation for liver cirrhosis patients. They formulated an AGP fucosylation index (AGP-FI) calculated as AGP fucosylation/AGP serum concentration and found that it is a useful tool for early detection of the disease. They found no significant difference in AGP fucosylation with regard to age. A sex related difference in AGP fucosylation was however reported by the same
team in 2002 [152]. Increased fucosylation of AGP in liver cirrhosis was reported to be due to increased fucosyltransferase activity in the liver [153].

Fucosylation of $\alpha_1$AGP was reported to be increased in RA [146, 154, 157]. It was found to decrease upon successful treatment with Methotrexate and fucosylation was thus suggested to reflect disease activity [158]. Increased fucosylation of haptoglobins, another glycosylated acute phase protein has also been detected in RA patients [159, 160]. Ryden et al. [155] studied the usefulness of AGP fucosylation as a marker of disease activity in RA. They noticed that it may be useful as a prognostic marker in men with RA whereas in women the hormonal factors might be interfering with advanced fucosylation of AGP and hence it can not be treated as can efficient marker.

Lauc et al. [161] in 1997 explained that a large increase in IgG fucosylation can not be explained by additional increase in fucose on N-linked oligosaccharides alone, but, fucosylation of O-linked glycans too is involved. An extensive study on IgG glycosylation in normal population showed that agalactosylated IgG (GO forms) are constantly increasing with age, especially in females [162]. This indicated an exhaustion of the glycosylation machinery that could contribute to less obvious increase in fucose content in older patients with RA than the juveniles as suggested by Jeddi et al. [163]. A 2.4-fold increase in the fucosylation of IgG heavy chains was reported in juvenile chronic arthritis [164]. Gornik et al. studied the fucosylation pattern of IgG in female patients with RA and observed a 40 % increase in fucose content with very high statistical significance [114].

The disease Leukocyte Adhesion Deficiency Type II (LAD II) discovered by Etzioni and Frydman was found to be a general fucose deficiency. These patients lack ABO blood groups containing the common Fucose $\alpha 1$-$2$ Gal
linkage. It was found due to the failure of conversion of GDP-mannose to GDP-fucose by GDP.D-mannose 4, 6 dehydratase in LAD II patients [91]. A deficiency in fucosylation may thus lead to many other diseases which are to be investigated in detail.

2.1.4.3. Hexosamines

Chu et al. studied the changes in the carbohydrate contents of glycosaminoglycans in the plasma of chicken induced for Tibial Dyschondroplasia (TD), a skeletal disorder seen in broiler chickens, ducks and turkeys [165]. Both glucosamine and galactosamine levels were elevated in TD severity. Glucosamine was elevated from 10 – 57% and galactosamine 9 – 48% as the TD score changed from healthy (1) to severe (4). The proteoglycans in the cartilage are chondroitin sulphate and keratan sulphate, the former containing galactosamine and glucuronic acid and the latter glucosamine and galactose. Elevated levels of monosaccharides in body fluids have been extensively documented in the diseases of articular cartilage [66-68]. These were considered to be derived from the degradation of proteoglycans in the matrix of connective tissue and sent for excretion through urine.

Lombart et al. [166] explained an increased incorporation of N-acetyl glucosamine, galactose and sialic acid into glycopeptides coupled with an enhanced activity of glycosyl transferase in terpentine induced inflammation of rat liver.

2.1.4.4. Sialic acids

Trabeisi et al. [167] elaborated the macrophage activation by mineral particles and subsequent alteration in glycosylation pattern. They noticed a decrease in the level of sialic acid in cells treated with MnO₂ and crystalline
quartz particles. No difference was noticed in fuscosylated structures between treated and untreated cells. The galactosylated structures increased in cells treated with crystalline quartz particles while it decreased in MnO$_2$ treatment. Phagocytosis of mineral particles by macrophages activated the latter leading to alterations in glycosylation brought about by different mechanisms:
i) difference in the level of expression of specific glycosyltransferases,  
ii) disturbing the enzymatic processing of the newly synthesized glycan as it passes from ER to trans-Golgi by affecting the cytoskeleton and motion of the organelle, iii) phagocytosis of particles stimulate release of lysosomal glycosidases that are able to degrade oligosaccharides. They have concluded that the changes in the carbohydrates on glycoproteins are critical in modulating macrophage function. This is supported by the findings of Pilatte et al. [131] that sialic acids are important molecules in the regulation of immune system and alterations in sialidase have pathophysiological implications in immunity.

Sialic acids are present in bacteria, absent in plants and are markers of species evolution in animals. Sialic acid content of glycoproteins appears to be responsible for their acidic properties. Those glycoproteins having the highest sialic acid content have the lowest isoelectric point. Sialic acids are of special mention among monosaccharides found in glycoconjugates. There are two reasons for this:

1. In eukaryotic glycoprotein glycans, they are always in terminal position on the glycan chain (except in a very few cases) conforming the role of recognition or anti-recognition signals they play.

2. The list of sialic acids is becoming long with around 36 types now. They differ in the substituents of the amino group (Acetyl or Glycolyl), in the
number position and nature (acetyl, lactyl or methyl) of substituents of hydroxyl groups of neuraminic acid [1].

Sialic acids play a role in conformation of glycans. The umbrella conformation of glycans is maintained firmly by ionic bonds between the electronegative charges of sialic acid residues and electropositive ones of basic amino acids. Removal of sialic acid residues makes the antenna free and consequently mobile, removes the protective effect of glycans and the glycoprotein becomes more antigenic and more susceptible to protease degradation.

Sialic acid of membrane glycoproteins inhibits platelet aggregation in vivo and in vitro [168, 169]. The content of sialic acid decreases with aging and in malignant conditions [170, 171]. In circulatory proteins, loss of sialic acid moiety targets them for degradation by liver cells which have asialoglycoprotein receptors [172]. Goswami and Kones [173] reported decreased levels of sialic acid in platelet proteins from diabetes, old age and Hodgkin’s lymphoma. In vitro treatment of platelets with free radicals was found to cause desialylation while addition of 2-mercaptoethanol, an antioxidant, significantly increased the level of sialic acid. Increased oxidative stress in the above clinical conditions might have involved in the desialylation of platelet glycoproteins. A contradictory result was obtained by Mezzano et al. [174] that sialic acid content does not differ in aged platelets raising a question about the significance of desialylation in physiological elimination of aged circulating cells.

Some workers have reported that level of free sialic acid in serum and / or urine was shown to be increased in malignancy and diabetes [175, 176]. This may be due to the cleavage of sialic molecules from circulatory and membrane
glycoproteins by glycosidases and their removal via excretion. Desialylation might be involved in altering cellular adhesion property leading to metastasis [177]. Increased levels of sialic acid in blood has been claimed to be associated with cataract [178] and cardiovascular diabetes [179].

Expression of a sialyl Lewis$^X$[$\text{NeuAc} \alpha 2-3 \beta \text{Gal} 1-4 (\text{Fucose} \alpha 1-3) \text{GlcNAc}$] portion in the carbohydrate chains of human AGP molecules has been a challenging target as related to inflammation [84]. The analysis of sialo and asialo-oligosaccharides of AGP as well as its glycoforms is important in understanding the biological roles of AGP [180].

Nakano et al. [85] reported distinct variations in the oligosaccharides of human, bovine, sheep and rat 1-AGP which all differed in the number of sialic acid-linked antennae. Human AGP contained di-, tri- and tetra antennary oligosaccharides with some tri antennary forms incorporating a fucose residue to form sialyl Lewis$^X$ structures [181, 182]. Rat AGP contained diantennary oligosaccharides as major type. On the other hand, bovine AGP contained sialo di antennary chains most exclusively. In sheep, mono and di antennary carbohydrate chains were abundant. Each sialic acid was attached to different positions (to Gal residue or to the GlcNAc residue of Gal – GlcNAc antenna). Sialic acids were attached to multiple sites of the core oligosaccharide and were not present as disialyl groups.

The type of sialic acid (N-acetyl or N-glycolyl neuraminic) and their presence in various organs of different animals, both young and adult have been studied by different workers [183-185]. Sialic acid is an important element in cell reactions and perception by its external environment [165]. Any qualitative or quantitative change in cell sialylation may modulate or alter certain cell functions.
2.2. GLYCOSIDASES

The quality control mechanism and the proper folding of glycoproteins rest in two membrane bound chaperones of rough endoplasmic reticulum. These are Calnexin and Calreticulin. Their role in binding the partly processed sugar chain GlcNAc$_2$Man$_9$Glc$_1$ obtained after glucosidase II digestion has been well established. They retain unfolded glycoproteins in the ER until they are correctly folded and assembled, an event which is signaled by the permanent removal of the terminal glucose residue by Glucosidase II. The folded glycoprotein is then transferred to cis-Golgi for further processing. Working with the Chinese Hamster ovary mutant M18-5 cells, Cancan et al. reported the presence of monoglycosylated glycans in the cytosol [186]. This indicated that part of the monoglycosylated glycoproteins can be directed into cytosol for deglycosylation process.

The lysosomal metabolism of glycoconjugates and the glycosaminoglycan degradation were elaborated by Winchester [23] as well as Kresse and Glossl [187]. Current knowledge regarding the role of specific glycosidases and glycosidic sulphatases in glycosaminoglycan catabolism primarily originates from studies of mucopolysaccharidosis [11, 188, 189], a disorder resulting from deficiency of the exohydrolase α-L-iduronidase. The catabolism of glycosaminoglycan begins with endohydrolysis of polysaccharides to oligosaccharides followed by the sequential action of an array of exoenzymes reducing these oligosaccharides to monosaccharides and inorganic sulphate. These oligosaccharides were elevated in urine samples from mucopolysaccharidoses I patients and were found to be useful biochemical markers for the identification and management of this disease [11].
Cantz and Kresse [190] established that fibroblasts isolated from patients with Sandhoff disease accumulate excessive amounts of glycosaminoglycans. Suzuki et al. [191] demonstrated that mice deficient in hexosaminidase isoenzyme developed a pathological situation like mucopolysaccharidosis. Articular cartilage from such mice were found to contain increased quantities of glycosaminoglycans [192]. These findings confirmed the notion that hexosaminidases play a key role in the degradation of glycosaminoglycans.

2.2.1. Glycosidase activity in diseases

The turnover of glycoproteins that play structural and cementing roles in eye lens was studied by Carlin and Cotlier [21]. They elucidated the characters of lens glycosidases involved in the breakdown of the carbohydrate portion of glycoproteins and glycolipids. Activity of six glycosidases namely β-glucosaminidase, β-galactosaminidase, β-galactosidase, β-glucosidase, α-mannosidase and β-fucosidase were assessed in the eye lens of human, rabbit and rat and the enzyme kinetics determined. Activities of these enzymes in rabbit lens, aqueous humor and serum were compared. Effect of inhibitors on glycosidase activity was also detected. Since lysosomes are absent in eye lens, these glycosidases sediment in the membrane fraction rather than the supernatant. Compared to other tissues, glycosidase activities in the crystalline lens were extremely low.

In another study, Carlin and Cotlier [22] measured the alterations in glycosidase activity in diabetic cataracts. Activities of the same six glycosidases in human autopsy lenses, senile cataract and diabetic senile cataract were compared. All enzymes except β-glucosidase and β-galactosaminidase showed significant increase in senile and diabetic cataracts. They also estimated the
glycosidase activities in kidneys of normal and alloxan diabetic rats. Activities of β-galactosidase and β-fucosidase decreased in diabetic rats while activities of other enzymes increased. They concluded that in diabetic mellitus, the tenuous equilibrium between the rate of synthesis and rate of degradation of glycoproteins is upset. It was likely that the increased glycosidase levels of diabetic rat lenses were insufficient to keep up with the elevated rate of glycoprotein synthesis.

Rellier et al. [31] reported that Advanced Glycation Endproducts (AGEs) modify galactose, fucose and sialic acid contents of cellular glycoproteins. They also investigated whether glucose and AGEs could affect the activities of glycosyltransferases (synthesis) and glycosidases (catabolism) in Bovine Retinal Endothelial Cells (BREC) and Bovine Retinal Pericytes (BRP). It was observed that high glucose concentration did not affect glycosidases and glycosyltransferases in either BREC or BRP with only exception of for β-galactosidase of BREC that showed increased activity. In contrast, AGEs increased β D-galactosidase, α L-fucosidase and neuraminidase activities in BREC and decreased galactosyltransferase, fucosyltransferase and sialyltransferase activities. In BRP, only galactosyltransferase activity was increased. No significant changes were observed for other enzymes. In the retina of diabetic rats, β D-galactosidase, α L-fucosidase and neuraminidase activities increased whereas fucosyl and sialyltransferase activities decreased.

Rellier et al. [31] concluded that the enzymatic glycosylation is dysregulated in diabetes and that AGEs are involved in the pathogenic mechanism. Though high glucose concentration did not affect the activities of the enzymes studied, AGEs influenced the activities differently in both the types of cells. A higher concentration of AGEs in vitro than those found in vivo may be one of the reasons
for this effect. In diabetic retina, the perturbations of enzymatic glycosylation observed were attributed to the disease state. They also suggested that the processes involved in the incorporation of $\beta$ D-galactose, $\alpha$ L-fucose and sialic acid into glycoconjugates (by glycosyltransferase activity) were reduced and that the catabolic mechanisms (glycosidase activity) were stimulated by exogenous AGEs.

The serum N-acetyl $\beta$-D glucosaminidase (NAG) activity was reported to be significantly higher independently of the concentration of serum glucose and glycosylated haemoglobin (HbA1c) in diabetic patients [15]. In the same study, there was no significant change in $\alpha$ L-fucosidase activity either in relation to HbA1c or to glucose levels. The increased activity of N-acetyl $\beta$-D glucosaminidase was believed to be due to the metabolic derangements in diabetes. In an earlier work, Whiting et al. [16] had reported elevated levels of N-acetyl $\beta$-D glucosaminidase in serum, urine and tears of diabetic patients. High serum NAG levels in diabetes have been postulated as being a compensatory mechanism for the increased deposition of mucopolysaccharides in the small vessels of diabetics and have been suggested as a good indicator of the onset of microangiopathy. The slightly increased urinary NAG levels found in diabetic nephropathy reflected early renal cell damage, correlated with elevated blood urea, but not with serum creatinine. Increased tear NAG levels were explained to reflect the compensatory mechanism in serum involving the deposition of glycoproteins in small vessels.

Skrha et al. [17] studied the changes of serum N-acetyl $\beta$-D glucosaminidase (NAG), tissue plasminogen activator (tPA) and erythrocyte superoxide dismutase (SOD) in relation to retinopathy in type I diabetes mellitus patients. A significant increase in mean serum NAG activity during four years was found only in patients without retinopathy, whereas no changes in the enzyme activity were present in patients with developing and established retinopathy. A significant positive
correlation was found between tPA and serum NAG levels. SOD activity was higher in the patients than in controls, but no difference was observed between patients with or without retinopathy. SOD also correlated positively with NAG level. They concluded that early functional changes such as activation of endothelial cells with a consequential increase of some enzyme activities may precede the morphological development of diabetic microangiopathy.

Tay-Sachs disease is a fatal cerebral degenerative disorder transmitted in an autosomal recessive manner and involves the accumulation of a specific ganglioside, GM₂. This compound possesses a terminal β-linked N-acetyl galactosamine residue. The absence of N-acetyl hexosaminidase A in tissue and body fluids of patients with Tay-Sachs disease could account for the ganglioside storage. O'Brien et al. [14] analyzed the levels of total hexosaminidase and hexosaminidase A in the children affected with this disease, their parents and unaffected family members. They realized that the percentage of Hex. A out of total hexosaminidase in the parents were in between the corresponding values for the affected children and the controls. Unaffected family members fell into 2 groups so far as percentage of Hex. A was concerned: the means and ranges of values of one group closely resembling the controls and of the other group resembling the values of parents of affected children. They concluded that the persons who are heterozygous for the gene for Tay-Sachs disease had reduced percentage of Hex. A in their serum than the healthy controls who are homogenous for this gene. The deficiency of this enzyme was the manifestation of the fundamental genetic defect resulting in Tay-Sachs disease. This assay may be useful in the detection of heterozygotes for a maker gene controlling a disease. Involvement of glycosidases in matrix destruction has been reported in Human Ovarian Carcinoma [193] and in experimental rat Osteocarcinoma [194].
A variety of acid glycosidases has been found in mammalian semen and secretions of male accessory sex organs. Among these, most active are N-acetyl β-D glucosaminidase, α-mannosidase, β-glucuronidase and β-galactosidase. Seminal acid glycosidases have not been studied in detail in birds. Droba and Droba [19] purified β-galactosidase from chicken seminal plasma and elucidated its properties. The purified fraction was homogeneous and had a Mol. wt. of 100 kDa. The pH optimum ranged from 3.6 to 4.0 with a pH stability from 5.5 to 7.5.

The presence of glycohydrolases in human serum is well established. But the cellular source of serum glycohydrolases is still unknown. It is suggested that seric glycohydrolases may be platelet derived [103]. Emillami et al. [195] surveyed the glycohydrolase activities in human platelets, evaluated their release upon stimulation with thrombin and characterized the most active enzyme β-N acetyl hexosaminidase. All the glycosidases occurred in the platelets with a wide range of activities. Among these, hexosaminidases had the highest specific activity. Incubation of the extracts of platelets with thrombin had no effect on glycohydrolase activities except of hexosaminidase which decreased by about 15%. An evaluation of the activities of glycosidases secreted by platelets in blood activated by thrombin produced by clotting blood was done by estimating these activities in human serum and, in parallel, in plasma from same donors, processed in a way that avoided platelet activation. The results clearly indicated lower activities in the plasma than in serum for hexosaminidase, α-fucosidase, β-galactosidase and β-glucosidase. The activity of α-mannosidase and α-galactosidase in serum and plasma did not differ significantly. However, among all these enzymes, β-glucosidase and β-galactosidase were having low levels of activity. They concluded that different
amounts of activity shown by each glycohydrolase might suggest the existence of various subpopulations of lysosomes containing different patterns of enzymatic activity. The secreted enzymes may play an important role in the degradation of components of the cellular environment and in the defense against infection.

2.2.2. Glycosidase activity in Rheumatoid Arthritis

A lysosomal mechanism of tissue injury in Arthritis was explained earlier by Weissmann [196]. He opined that some materials in the lysosome can provoke inflammation, tissue injury and breakdown of connective tissue. Studies had shown that local lesions of RA are characterized by the margination of leukocytes and their appearance in the synovial fluid, hypertrophy and hyperplasia of synovial lining cells, presence of abnormal numbers and configuration of lysosomes, infiltration of synovium by many lymphocytes frequently in clusters, transformation of synovium into granulation tissue, erosion of cartilage initially by the matrix followed by chondrocyte death and attempts for regeneration. Analysis of synovium and synovial fluid from RA patients has documented considerable increase in lysosomal enzymes [197].

Rheumatoid arthritis is characterized by damage to the cartilage of joints. Cartilage being formed of proteoglycan aggregates constrained within a net work of collagen, any degradation to this matrix material will lead to severe damage. Lysosomal glycosidases are capable of hydrolyzing oligosaccharides of proteoglycans of cartilage. The exoglycosidases cleave the terminal monosaccharide moiety whereas the Endoglycosidases attack the interior glycosidic (hexosaminidic) linkage. The exoglycosidases like $\beta$-N acetyl hexosaminidase, $\beta$-glucuronidase, $\beta$-galactosidase, $\beta$-glucosidase, $\alpha$-mannosidase
and α-L-fucosidase have been subjects of study for many researchers working on glycoprotein catabolism in RA.

Kar and Pearson [198] reported an increase in the activities of β-N acetyl glucosaminidase and Arylsuphatase A in the serum of patients with various inflammatory disorders of muscle and connective tissue like Polymyositis, Dermatomyositis, Systemic Lupus Erythematosus and in a few cases of other systemic collagen diseases. They recorded significant increase in β-acetyl glucosaminidase activity in sera of patients with Rheumatoid Arthritis. Reviewing similar studies of other workers, Kar and Pearson have concluded that the elevated levels of this enzyme activity is one biochemical feature which is common to several diseases of that group.

Vijayalakshmi et al. [32] studied the effect of milk extract of *Semocarpus anacardium* nuts on glycohydrolases and lysosomal stability in adjuvant arthritis in rats. Their results showed significant increase in plasma glycoprotein level in arthritis as evidenced from the increased concentration of plasma protein bound hexoses, hexosamines and sialic acid. The total and free activities of lysosomal enzymes (β-glucuronidase, β-galactosidase, β-N acetyl glucosaminidase, Cathepsin and Acid phophatases) which increased significantly in arthritic condition was significantly reduced by drug treatment. All the tissues (liver, kidney and heart) and the plasma examined behaved in the same manner. The glycoprotein level was also reduced significantly by the extract treatment. The metabolism of glycosaminoglycans and glycoproteins is altered due to the increased release of acid hydrolases from liposomes during arthritis and the drug exerted its antiarthritic activity through lysosomal enzyme stabilization. This conclusion was supported by the works of Reddy and Dhar on degradation of proteoglycans [133], of Fraser *et al.* [199] and Lombart *et al.* [166] who too
reported enhanced activity of sialyltransferase and galactosyltransferase in serum and liver of inflamed rats.

Lysosomal exoglycosidases participate in the destruction of the articular cartilage by cleaving glycoside bonds in glycoproteins and proteoglycans. Pancewicz et al. determined the activity of the exoglycosidases namely, hexosaminidase, β-glucuronidase, β-galactosidase, α-mannosidase and α-fucosidase in serum and synovial fluid of patients with Lyme arthritis (LA), Juvenile idiopathic arthritis (JIA) and Rheumatoid arthritis (RA) using the p-nitrophenyl derivatives of sugars as substrates. A significant increase of the activity of all the exoglycosidases in serum and in synovial fluid of the patients with different forms of arthritis was found. The ratio of synovial fluid/serum activity of exoglycosidases was above 2.0 in LA, but not in JIA and RA patients. As the main source of exoglycosidases in the joint is the synovial membrane, this result supported the appropriateness of therapeutic synovectomy in chronic Lyme arthritis with knee effusion. The serum activity of hexosaminidase may be used in monitoring the course of Lyme arthritis and the efficiency of treatment [200].

Berenbaum et al. [201] reported marked elevation of N acetyl β-D hexosaminidase (NAHase) activity in the sera and synovial fluid of RA patients. The activity of this enzyme in the medium containing rabbit articular chondrocytes stimulated with IL-1β was dramatically higher than unstimulated medium of primary culture. This suggested that cartilage (chondrocytes) is the source of serum and synovial fluid NAHase activity in RA joints. Over production of this enzyme from the joints could degrade the matrix leading to complications. The possibility of NAHase as a marker of erosion that is independent of the inflammation status has to be explored.
The enzymatic activities of 8 key glycosaminoglycan degrading glycosidases and glycoside sulphatases in cultured human articular chondrocytes and in synovial fluid from osteoarthritic patients were studied by Shikhman et al. [20]. Hexosaminidase was found to be the most dominant enzyme released by the chondrocytes and in the synovial fluid. Stimulation of chondrocytes with interleukin-1β (IL-1β) resulted in a selective increase of extra cellular hexosaminidase activity and to a lesser degree, of the extra cellular β-galactosidase activity, without significant changes in the activity of other studied enzymes. They concluded that lysosomal glycosidases, particularly hexosaminidase, represent a distinct subset of cartilage matrix-degrading enzymes that are activated by proinflammatory stimuli.

Application of enzyme inhibitors as new drug candidates for the therapy of osteoarthritis was explained by Liu et al. [202]. They synthesized and investigated a series of iminocyclitols which inhibited hexosaminidase activity in human or cultured articular chondrocytes and human chondrosarcoma cells. This inhibition resulted in glycosaminoglycan accumulation in chondrocytes and cartilage. The impaired catabolism of glycosaminoglycans was reversed by addition of hexosaminidases.

It was established that a large amount of hexosaminidase activity in human serum originated from platelets and the blood platelet count in RA patients was often increased [193]. Casal et al. [33] investigated the relationship between plasma activity of hexosaminidase and disease activity or severity. They noticed that enzyme activity in plasma was lesser than serum and the former increased significantly after platelet stimulation. A significant correlation was found between the difference of serum/plasma hexosaminidase activities and the blood platelet count. Their result also showed that although
the hexosaminidase activity in serum is significantly higher than in plasma, its isoenzyme composition was similar in both biologic environments. They constituted a significant correlation between the activity of plasma Hex. B isoenzyme and the number of inflamed joints. This finding contradicted the report of Berenbaum et al. [198] that the hexosaminidase activity in serum is independent of inflammation status in RA patients.

Hexosaminidase is the major glycosidase expressed within the joints, and it is primarily produced by synovial fibroblasts. Hex A subunit gene, one of the two genes encoding for the alpha or the beta chains of hexosaminidase, was characterized by the strongest gene expression. It was followed by the expression of Hex B subunit gene and the β-D glucuronidase gene. According to Pasztoi et al., glycosidases expressed by synovial membranes and synovial fibroblasts are under negative regulation by some locally expressed cytokines both in rheumatoid arthritis and osteoarthritis. This does not exclude the possibility that these enzymes may contribute significantly to cartilage degradation in both the joint diseases if acting in collaboration with the differentially upregulated proteases to deplete cartilage in glycosaminoglycans [203].

Multiple isoforms of mammalian alpha-mannosidases are active in the pathways of N-linked glycoprotein synthesis and catabolism. They differ in specificity, function and location within the cell and can be selectively inhibited by imino sugar monosaccharide mimics. Two related compounds were shown to be potent inhibitors of lysosomal alpha-mannosidase with different potencies towards alpha1,6 mannosidase. The availability of more selective inhibitors allows the pathways of N-linked oligosaccharide metabolism to be dissected [204].
2.3. ANTIOXIDANTS AND GLYCOPROTEINS

2.3.1. Antioxidants in diseases

Metabolism like other aspects of life, involves trade offs. Oxidant by-products of normal metabolism cause extensive damage to DNA, protein and lipid. This damage may contribute to aging and to degenerative diseases of aging like cancer, cardiovascular disease, immune system decline, brain dysfunction and cataracts. Ames et al. [37] has reviewed the oxidative damage to DNA, protein and other macromolecules and have related these damages to aging. It is believed that the level of oxidative DNA damage is roughly related to metabolic rate in a number of mammalian species. Super oxide (*O₂⁻) and hydroxyl radical (*OH⁻) which are mutagens produced by radiation are also byproducts of normal metabolism. Lipid peroxidation also gives rise to mutagenic lipid epoxides, lipid hydroperoxides and other reactive radicals. Singlet oxygen, a high-energy mutagenic form of oxygen can be produced by transfer of energy from light, the respiratory burst of neutrophils or lipid peroxidation. All these cause damage to DNA [37].

Oxidatively damaged DNA is repaired by enzymes that excise the lesions and are then excreted via urine. Methods have been developed to assay several of these excised and damaged bases in the urine of man and rodents [37]. They accumulate with age. Mutations were also found to be accumulating with age. The existence of specific repair glycosylases that excise the lesions from DNA has been reported in cancer and aging. All these observations reveal that oxidative damage to DNA has senile and pathological impacts.

Proteins and lipids are also damaged by endogenous oxidants. It is reported that the activity of proteolytic enzymes that hydrolyze oxidized protein
is not sufficient to prevent an age-associated increase of oxidized proteins. In the two cases of premature aging in man, Werner Syndrome and Progeria, oxidized proteins increase at a much higher rate than is normal. The concentration of certain fluorescent pigments produced by cross links between protein and lipid peroxidation products also increased with age [205].

Ames et al. [37] has highlighted the antioxidant defense offered by ascorbate, tocopherol and carotenoids. They have assessed that low dietary intake of these substances double the risk of most types of cancer, heart disease and cataracts caused by oxidative stress.

The activity of SOD and the level of malondialdehyde in the plasma of patients with acute appendicitis were measured by Koltuksuz et al. [206]. Both SOD and MDA levels were found to be significantly higher in acute appendicitis groups compared with controls. They opined that Oxygen Free Radicals (OFR) play an important role in the extent of acute appendicitis and that organism may increase SOD and other antioxidant enzyme levels to prevent the hazardous effects of OFR.

Antioxidant parameters have been documented in different types of lung diseases implied with occupational hazards. Mechanisms of cell injury and repair are well explained in the lung. The reactive oxygen species are secreted by various types of inflammatory cells with the alveolar macrophages playing a key role in this process [207]. Cantin and Crystal [208] identified that tissue alterations like lung fibrosis may be due to oxidative damage caused to proteins, carbohydrates and lipids peroxidized by ROS. Borm et al. [209] reported higher red blood cell reduced glutathione (GSH) levels in silicotic patients as compared to healthy controls and later established a strong correlation of SOD and GPx with the disease [210]. Eugelen et al. [211] studied
pneumoconiosis in local workers and proved that the levels of SOD, GSH and catalase were significantly decreased in the RBC of workers with pneumoconiosis. This decrease was suggested to be due to the excessive release of Reactive Oxygen Species by alveolar macrophages and neutrophils in the lung tissue.

Nadeem et al. [212] have observed altered levels of antioxidants in Chronic Obstructive Pulmonary Disease (COPD). Measuring a wide range of parameters of oxidant-antioxidant balance in leucocytes, plasma and red cells of COPD patients and healthy non smoking controls, they reported that activities of Glutathione peroxidase (GPx) was lowered, SOD increased and catalase remained unchanged in red cells of patients compared to control. In plasma, GPx and lipid peroxidase were higher in patients. Plasma total nitrates and nitrites were almost the same in both the groups.

Studies have indicated that antioxidant enzymes play a role in the defense of mammalian lung tissues from damage incurred by exposure to ozone. Morgan [213] established a protective role for catalase and SOD in cultured rat lung fibroblasts. Acute exposure to ozone caused an initial glutathione (GSH) depletion in lung tissue, whereas continuous exposures resulted in restoration of GSH levels within days. This increase correlated with increases in GPx and GSH reductase [214]. Rahman and colleagues [215] measured antioxidant enzyme mRNA and enzymic activity in whole lungs of rats exposed in vivo to 0.7 ppm ozone for 5 days. SOD activity was increased 2 fold whereas catalase and GPxase showed subtle rises in activity. In another study by the same group [216], antioxidant enzyme response to ozone exposure in rat heart and brain was examined. The level of thiobarbituric acid-reactive substances (TBARS), Glutathione peroxidase and catalase activities increased in both the tissues.
These findings indicated that ozone concentrations found in some urban areas resulted in the pathogenesis of some forms of heart and brain diseases [217].

Hyperoxia-induced lung damage is of great clinical interest due to the use of oxygen therapy in the care and management of many patients. Specific targets of hyperoxic insult to the lung appear to be the epithelial cells of alveoli and the vascular endothelial cells which are prone to active oxygen species. Increased production of antioxidant enzymes in these cells under hyperoxia has been explored [218].

In rat neonatal lung, increased activity of SOD has been related to an increase in mRNA for this enzyme [219]. Rat airway epithelial cells on the other hand were shown to be resistant to hyperoxia by possessing constitutively higher levels of antioxidant enzymes than the other lung parenchymal cells [220]. Mice transfected with the human MnSOD gene showed 40% increase in this enzyme activity in whole lung homogenates compared to transgene negative mice [221].

Exposure of cultured epithelial cells to 95% oxygen for 3 days resulted in elevated mRNA levels and enzyme activities of CuZnSOD and Glutathione peroxidase; an increase in mRNA for catalase, but a decrease in its activity; and no change in mRNA levels of MnSOD [222]. After 5 days’ exposure, mRNA levels for all antioxidant enzymes increased, activities of Glutathione peroxidase and CuSOD were elevated, but catalase activity decreased even further and MnSOD remained unchanged.

Increases in antioxidant enzymes in response to mineral dusts (asbestos and silica) has been reported by various workers [223-225]. The increase in enzyme activity correlated with the increased mRNA expression for these
enzymes, the exception being CuZnSOD which showed no statistically significant increase over the control values. However, it appears doubtful that increases in antioxidant enzymes in response to mineral dusts at high concentrations are sufficient to protect the lung from oxidative damage.

Oxidative stress induced by cigarette smoking has been a widely investigated problem. One puff from a cigarette contains approximately $10^{15}$ oxidant radicals in the combined gaseous and particulate states. Reactions with smoke prolongs half-lives for many of these radicals. Alveolar macrophages isolated from smokers and smoke exposed hamsters exhibited more than a 2-fold increase in the activities of total SOD and catalase [226] with no corresponding increase in Glutathione peroxidase activity. Red blood cells from smokers contained more glutathione and catalase than from non-smokers, but the Glutathione peroxidase levels were the same in both the groups [227].

The regulation of antioxidant enzymes in lung exposed to oxidative stress has been reviewed in detail by Quinlan et al. [50]. Different agents produce ROS by different mechanisms. The damage to lung tissue also varies. Also, the activities of antioxidant enzymes are influenced differently. The cloning of genes encoding antioxidant enzymes and the development of shuttle vectors enabling over-expression of these enzymes in cells in vitro are exciting recent findings with implications for gene therapy. Development of synthetic scavengers of ROS and techniques for more effective targeting of cells will allow both preventive and therapeutic approaches to oxidative stress.

Cataracts have an oxidative etiology and dietary antioxidants can prevent their formation in man. Dietary ascorbate, tocopherol and carotenoids have protective effect on cataract. Smoking, a severe oxidative stress is a major risk
factor for cataract. Radiation, an oxidative mutagen is known to cause cataract [37].

Plasma and erythrocyte levels of SOD, Catalase and GSH were found significantly lowered in patients suffering from non insulin dependent Diabetes mellitus [228]. Changes in antioxidant levels were correlated with the duration of the disease and with the development of complications.

Oxidative reactions play a central role in atherogenesis [205]. Endothelial cell dysfunction may be related to an increase in oxidative stress. It has been shown that cardiovascular disease is associated with low plasma concentrations of ascorbate, tocopherol and β-carotene [229]. Serum carotenoids and these vitamins are accepted as markers of endothelial dysfunction. Oxidative modification of apolipoprotein B 100 plays a key role in recognition of low density lipoproteins (LDL) and that LDL uptake by scavenger receptors in macrophages leads to foam-cell formation and atherosclerotic plaques. Oxygen free radicals and lipid peroxidation are major factors in the etiology of atherosclerosis and its associated clinical disorders like coronary artery disease, Stroke, Ischemic dementia etc. [44, 230]. Free radicals are thought to play a major role in reperfusion injury. Many free radical scavengers and antioxidants have been shown to reduce reperfusion injury [231].

Cell division itself is a key factor in mutagenesis. Increased rate of division in Stem cells often make them precursors of these tumour cells. Increasing their cell division rate would increase mutation brought by an unpaired DNA segment. Oxidants stimulate cell division as has been noted in inflammatory processes accompanying wound healing [37]. Antioxidants are found to decrease mutagenesis and thus carcinogenesis in two ways: by decreasing oxidative DNA damage and by decreasing cell division. Tocopherol
and carotenoids can prevent cell division and hence reduce the risk of cancer. Two of the major causes of cancer – cigarette smoke and chronic inflammation – appear to involve oxidants in their mechanism of action. Research on the relationship between antioxidant levels and cigarette induced cancer has showed a statistically significant protective effect of antioxidants. Inflammatory reactions release large amounts of NO, a nitrosating agent and indirect mutagenic oxidant. Ascorbate inhibits nitrosation under physiological conditions. Thus antioxidants help to protect against carcinogenic effects of chronic inflammation [37].

Biochemical studies have shown that oxidation may be important in a number of brain pathologies [36]. Ischemic episodes of brain like Parkinson’s disease liberate iron, an important catalyst in the reactions forming oxygen radicals which brings oxidative stress [232, 233] and low GSH and Glutathione peroxidase levels in the substantia nigra [234]. In individuals suffering from this disease, the oxidative DNA damage is elevated. The protective role of SOD against brain injury due to ischemia is supported by the finding that it’s over-production is protective. Alzheimer’s disease is explained due to an enhanced oxidative stress as evidenced by increased levels in the activities of SOD, Catalase and Glutathione peroxidase [64] and also due to reduced plasma levels of the antioxidant micronutrients like Vitamins A and E and Carotenoids [235]. Senile plaques seen in Alzheimer’s disease contain aggregates of β-amyloid protein which was found to play an important role in oxygen radical generation [236, 237]. The gene for the production of Amyloid Precursor Protein (APP) is on the 21st chromosome and the anomaly in this chromosome defined as Downs Syndrome often gets associated with Alzheimer’s dementia [46, 236].
Montoliu et al. [238] analyzed the effect of chronic and in vitro ethanol exposure of brain oxygen radical formation and lipid peroxidation in rats. Ethanol induced a dose-dependent increase in lipid peroxidation in brain homogenates. This treatment produced a decrease in GSH / GSSH ratio in brain and significantly enhanced the levels of SOD and catalase activities. They concluded that this mechanism could be involved in the toxic effect of ethanol on brain and alterations occurring after chronic ethanol intake.

The activity of SOD, Catalase and Glutathione peroxidase was studied in different regions of brain of young and old rats by Rani and Paneerselvam [239]. They evaluated the lipid peroxidation and the activities of SOD, Catalase and Glutathione peroxidase in different regions of brain in young and old rats. Lipid peroxidation was higher for old rats in all brain regions than for young control. Activities of antioxidant enzymes was lower in most of the regions of brain of old rats. Administration of L-Carnitine reversed the age associated changes in a duration-dependent manner. The neuroprotective effect on brain in old rats was achieved by the elevation of antioxidants with L-Carnitine.

Oxidative stress leads to birth defects and childhood cancer [37]. Oxidative lesions in sperm DNA are increased 250% when levels of dietary ascorbate are insufficient to keep the seminal fluid ascorbate at an adequate level. The oxidants in cigarette smoke will deplete the antioxidants in plasma. A comparative study on sperms from smokers and non-smokers showed that the number of sperms and the percentage of motile sperms decreased significantly in smokers and this decrease was dependent on the dose and duration of smoking. Paternal smoking increased the risk of birth defects and childhood cancer in their offspring. Inadequate diets and smoking of fathers appear to
result in damage not only to their own DNA, but also to the DNA of their sperm affecting future generations.

It has recently been shown that Metallothioneins (MTs) play a direct role in cellular defense against oxidative stress by acting as antioxidants. MTs are cystein-rich, low molecular weight, metal binding proteins found in a variety of organisms ranging from bacteria to man and were believed to be involved in reducing metal toxicity. Koh and Kim studied the effect of MT on the activity of antioxidant enzymes SOD, catalase and peroxidase [240]. It was reported that SOD activity was increased in presence of MT, but decreased in the presence of an Apo-MT (MT from which the metal group was released). At the same time, MT decreased the activities of catalase and peroxidase. The function of MT as an antioxidant might effect the level of superoxide scavenging and not the level of hydrogen peroxide. The physiological role of co-operation of SOD and MT in cells exposed to superoxide remains obscure.

Ramanathan et al. studied the effects of ascorbic acid and α-tocopherol on arsenic induced oxidative stress in rats [241]. Arsenic treated rats showed elevated levels of lipid peroxidation, decreased levels of non-enzymatic antioxidants and activities of enzymatic antioxidants. Ascorbic acid and α-tocopherol treatment reduced the level of lipid peroxidation, enhanced the levels of glutathione (GSH). Ascorbic acid and α-tocopherol also enhanced the activities of SOD, Catalase, Glutathione peroxidase and Glutathione reductase.

2.3.2. Antioxidants in Rheumatoid Arthritis

Free radicals or Reactive Oxygen Species play an important role in inflammation. During the process of phagocytosis, inflammatory cells produce (\(*O_2^-\)) which are dismutated into \(H_2O_2\). The cell and tissue injury associated
with acute and chronic inflammation are attributed to the oxidative stress caused by these radicals [242]. The nitric oxide (NO) contribute to cardinal signs of inflammation, superoxide radical (O2·-) to fibroblast proliferation and hydrogen peroxide (H2O2) to the activation of transcription factors including IL-2 and TNF-α. Other control mechanisms that are perturbed in inflammation are: DNA damage, lipid peroxidation and heat shock protein production by the activation of neutrophils.

RA is a systemic disease characterized by progressive, erosive and chronic polyarthritis [39]. Cellular proliferation of the synoviocytes and neoangiogenesis lead to the formation of pannus which destroys the cellular cartilage and the bone [243]. In RA, the immune complexes activate the neutrophils with subsequent production of superoxide anion radicals. SOD then converts superoxide into hydrogen peroxide. Most of this H2O2 is inactivated by GSH peroxidase and catalase, but some of it remains and is converted to hydroxyl radicals by iron catalysis [44]. A recent study indicated that increased oxidative stress and/or defective antioxidant status contribute to the pathology of RA [244]. It was reported that the malondialdehyde level increased and the level of endogenous antioxidants was lowered in RA patients. Plasma catalase activity has been reported to be lower in patients with RA [245] and glutathione reductase activity reduced in synovial fluid [246]. Increased oxidative stress and decreased levels of antioxidants have been reported in active RA [247] and juvenile idiopathic arthritis [248]. RA has been characterized as a free-radical produced disease [249]. A decrease in antioxidant status has been reported in RA by Nalini et al. [250].

RA is accompanied by abnormalities in body iron metabolism. Gutteridge [251] reported a rapid fall in the iron content of blood plasma at the onset of
inflammation followed by an increased deposition of iron proteins in the synovial fluid. In the synovial fluid of inflamed joints, the iron released during necrosis might catalyze the formation of OH⁻ (hydroxyl) radicals from H₂O₂, thus contributing to an increase in inflammation. Increased deposition of iron in synovial membrane, a drop in blood haemoglobin and the presence of iron complexes catalytic for radical reactions in synovial fluid have been reported by many workers [252, 253]. The extensive deposition of ferritin iron within synovial membranes with inflamed joints would increase its sensitivity to radical reactions; H₂O₂ generated by phagocytes could easily penetrate into the synovial cells and react with iron mobilized from ferritin to form OH* radicals. OH* radical formation could account for some or all of the hyaluronic acid depolymerization [254] and cartilage degradation seen in rheumatoid joint [255].

The singlet molecular oxygen is involved in the breakdown of collagen. The proinflammatory roles for hydrogen peroxide and lipid peroxides and a possible involvement of hydroxyl radicals and singlet oxygen in the breakdown of collagen is supported by Bragt et al. [256]. They have also reported that a combined administration of catalase and SOD was more effective than the administration of catalase alone. Oyanagui [38] suggested that suppression of inflammation by the anti-inflammatory drug Dexamethasone was enhanced by the co-administration of antioxidants like α-tocopherol, β-caroten etc. This finding helped to replace the use of expensive immunosuppressants when the antioxidants effectively prolong the action of anti-inflammatory drugs.

Alkyol et al. [257] studied the relationship between plasma and erythrocyte antioxidant enzymes and lipid peroxidation in patients with RA. They reported that there was no significant difference in the activities of erythrocyte enzymes between patients and controls. Recently, Bae et al. [258] conducted a cross-sectional case-
control study to estimate the antioxidant nutrient intake by RA patients and correlated it with plasma antioxidant status. They reported that the intake of calorie as well as vitamin A and β-carotene were significantly lower in patients than those of the controls. Patients had a significantly lower plasma α-tocopherol level. Plasma SOD and GPx activities were significantly lower in RA, being 62% and 80% respectively of those in controls. However, plasma MDA concentration was slightly higher in RA patients. They concluded that decreased levels of plasma antioxidants and decreased activity of antioxidant enzymes are due to either the inadequate antioxidant nutrient intake or the active inflammatory disease itself. In another study on RA patients, it was observed that the plasma levels of Vit. C, Retinol and Uric acid inversely correlated with the variables related to disease activity. It was suggested that proper dietary antioxidant nutrient intake may reduce free radical generation and improve antioxidant status in RA [259].

Studies have shown that the markers of antioxidant status in the blood of RA patients are lower than those of their controls. The decreased antioxidant status of RA patients may be explained as a result of excessive need for antioxidants during inflammation. Morgan et al. [260] had indicated that antioxidant nutrient intake and plasma levels are not optimal in RA patients. Nutrient intake of antioxidants in Juvenile Arthritis patients was also shown to be different from that of healthy counterparts [261]. Arajuo et al. [262] had implied a decreased level of vitamin E as a possible cause of development of Juvenile Rheumatoid Arthritis in children. Trap et al. [263] proved that concentration of blood selenium (a component of GPx) was lower in RA patients than the controls and that selenium supplementation increased blood GPx activity in these patients. Knekt et al. [264] also suggested that low selenium status may be a risk factor for rheumatoid factor-negative RA whereas
low α-tocopherol status be a risk factor for RA independently of RA factor status. Investigations have elucidated that many aspects of RA (rheumatoid factor (RF) positivity, severity, and so forth) can be linked to smoking. Recent data suggest that cigarette smoking establishes a higher risk for anti-citrullinated protein antibody (ACPA)-positive RA. [265]. This had earlier been attributed to the oxidative stress induced by cigarette.

Recent studies using animal models have suggested an anti-inflammatory role for antioxidants like SOD [266] and vitamin E [267]. A new strategy of antioxidant therapy for the prevention and treatment of RA was involved in some studies [268, 269]. Vitamin E seems to uncouple joint inflammation and joint destruction in transgenic mouse model of RA and offers beneficial effects on joint destruction [270]. Serum concentrations of vitamin E, retinol and β-carotene were suggested to be the possible risk factors for developing RA [271]. Edmonds et al. [272] reported that clinical symptoms improved with the supplementation of vitamin E at a dose of 600 mg/day. Halevy and Sklan [273] opined that the possible mechanism by which vitamin E alleviated RA symptoms was a reduction in the formation of prostaglandins, major molecules produced during the inflammatory process.

Certain other dietary substances capable of affording protection or modulating the onset and severity of arthritis have been studied for their antioxidant effect. Inhibition of CIA has been reported in taxol-treated rats with a reversion of affected synovium into naïve form [274]. Rolipram, a type IV phosphodiesterase inhibitor has been shown to ameliorate CIA by suppressing the expression of Tumour Necrosis Factor α (TNF-α) in mice. TRK-530, a newly synthesized derivative of disphosphonate is reported to inhibit the development of CIA in mice. The polyphenolic fraction isolated from green tea (GTPs) too
possesses anti-inflammatory as well as anticarcinogenic properties. The arthritic index was found to be significantly lower in GTP fed collagen-induced arthritic mice than the non GTP fed counterparts. They also showed marked reduction in the expression of inflammatory mediators like cyclooxygenase-2, IFN-γ and TNF-α in arthritic joints of GTP fed mice. Histological and immunohistochemical analysis of arthritic joints in GTP fed mice demonstrated only marginal joint infiltration by IFN-γ and TNF-α. These anti-inflammatory roles were attributed to the antioxidant effect of green tea which could be recommended as an oral supplementary diet or drink [274].

Oyanagni [38] reported that the natural antioxidants like bilirubin, β-carotene, flavanoids like rutin and morin, melatonin, ascorbic acid and α-tocopherol enhanced the immunosuppressant effect of Dex (Dexamethasone, 0.1 mg / kg, s.c.) as revealed by about 30% suppression of ischemic paw oedema in mice. When tested alone, these antioxidants could not effect more than 10% suppression, even at higher doses. Clinical trials on non toxic natural antioxidants co-injected with glucocorticoids offered improved results with very little side effects in alleviating inflammation.

It has been widely accepted that the inflammation process produces free radicals, thereby decreasing SOD activity. DiSilvestro et al. [275] substantiated this hypothesis by showing that administration of anti-inflammatory drugs increases plasma SOD activity. The antioxidative and immunosuppressive effects of the drug Allopurinol, commonly used for treatment of gout and other inflammatory conditions were investigated by Miesel et al. [276]. Unlike conventional antirheumaic therapy with NSAIDs (Non Steroidal Anti Inflammatory Drugs) and SAARDs (Slow Acting Anti Rheumatic Drugs) which is often complicated by loss of effectiveness and severe side effects, daily doses of
Allopurinol (100–900 mg) are well tolerated by most of the patients. Most common antirheumatic drugs act also by modulating the levels of reactive oxygen species, and serve as important mediators and signal transducers in inflammatory and autoimmune diseases. Allopurinol too modified the oxidative burst of phagocytes, inhibited xanthine oxidase and displayed immunosuppressive effect in RA patients [276].

Efficacy of antioxidants as adjuvant therapy was investigated by Helmy et al. [269] who showed that combination of standard treatment and antioxidants increased serum GPx activity with better disease control. A curative effect of vitamin E was implied in this work. Thabrew et al. [277] concluded that increases in serum SOD and GPx activity in RA patients treated with antioxidant polyherbal preparations resulted either from transcriptional activation of these enzymes or removal of oxidative stress.

Nalini et al. [278] studied the effect of pre and post treatment with Type II collagen on the antioxidant status and the circulating immune complex (CIC) level in Adjuvant Induced Arthritic (AIA) rats. They reported a significant decrease in the level of ascorbic acid (ASA), reduced glutathione (GSH), superoxide dismutase (SOD) and an increase in lipid peroxidation in the spleen and thymus of AIA rats. Pretreatment with Type II collagen brought significant changes in all these parameters towards normal values. The need for using phytochemicals as antioxidants against diseases caused by free radicals is highlighted in the review by Sen et al., 2010 [279].

A number of spice principles like Curcumin (from turmeric), Eugenol (from cloves), Capsaicin (from red chilly) and Zingerone (from ginger) have shown inhibitory action on lipid peroxidation in a dose-dependent manner [280]. These spice principles showed great differences among themselves in
their effect on lipid peroxidation. Curcumin, Eugenol and Capsaicin inhibited lipid peroxidation at low concentration (25-60 µM) while Gigerone required a higher concentration (450 µM) to show 50% inhibition. It was also shown by this study that the inhibition of lipid peroxidation by Curcumin and Eugenol was reversed by adding high concentrations of Fe\(^{2+}\). They concluded that spices may exert their effects on lipid peroxidation by scavenging reactive oxygen species and this necessitated a chelation of metal ions as had been suggested by earlier investigators [281-284]. The antioxidant role of Curcumin or Turmeric has been elaborated by different workers [281-287]. Jackson et al. [288] recently reported that Curcumin inhibited neutrophil activation, synoviocyte proliferation, angiogenesis and collagenase expression in arthritis. This was attributed to the antioxidant role of this agent.

2.3.4. Glycoproteins and antioxidants

Oxygen derived free radicals not only suppress proteoglycan synthesis [289, 290] but also enhance proteoglycan degradation in articular cartilage [291]. It has been reported that in articular cartilage treated with hydrogen peroxide, the proteoglycan monomer loses its capacity to interact with hyaluronic acid without getting degraded [292, 293]. The enhanced release of proteoglycans from the X-ray irradiated cultures was attributed to the effect of free radicals on proteoglycans [294].

The role of antioxidants in the suppression of certain glycoproteins has been studied by different workers. Levels of Cytokines such as TNF-\(\alpha\), IFN-\(\gamma\) and Interleukins were found elevated in inflammatory joints [295]. In CIA rats fed with GTP, their levels were reduced to normalcy due to the antioxidant effect of green tea [275]. C-reactive proteins (CRP) which was increased in endothelial inflammation was reduced by vitamin C and vitamin E because of
their antioxidant effect [229]. On contrary to these, Mathy-Hartert et al. [296] stated that ROS may have anti-inflammatory properties by depressing inflammatory gene expression and that these effects are dependent on the nature of radical species and the signaling pathway that is activated. They also alarmed that these findings are to be considered for management of antioxidant therapy against inflammatory joint diseases.

The levels of sialic acid and carboxylation in platelet proteins from diabetes, old age and Hodkin’s lymphoma cases were evaluated by Goswami and Koner [173]. The levels of sialic acid residues in platelets was found to be significantly lower and carboxylation of proteins (oxidative damage) was higher in these conditions compared to controls. Addition of 2-mercaptoethanol, an antioxidant, significantly attenuated the above effects. The study indicated the possible role of free radicals in desialylation of platelet protein in these clinical conditions.

The literature reviewed in this chapter is mainly related to the alterations in the carbohydrate components of glycoproteins in plasma or serum and synovial fluid during inflammations. Similarly, studies on glycosidases were limited to the activity of hexosaminidase and a few of the other glycosidases individually or separately taking plasma or serum and chondrocytes as the sources. Investigations on the variation of monosaccharide concentration and a detailed survey on glycosidases in other tissues like liver, heart, kidney, testes, and brain of RA patients are scanty. No studies on the changes in the carbohydrate components of glycoconjugates in association with oxidative stress or with antioxidant treatment have been made so far. A correlation between glycans and carboxylation of proteins in plasma or tissues in pathological conditions may probably widen the scope of using antioxidants in the therapy of many of the diseases.