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REVIEW

Vitamin D-binding protein as a biomarker to confirm specific clinical diagnoses

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ABSTRACT

Introduction: Vitamin D-binding protein (DBP) performs a variety of functions as a transporter for various ligands and takes part in a number of systemic and local physiological and pathological processes. The knowledge about the pathomechanisms of this protein involvement justifies its use as a biomarker to confirm specific clinical diagnoses suggested by nonspecific signs and symptoms.

Areas covered: DBP has properties of both systemic laboratory parameters measured in the blood plasma and specific parameters measured in variety of physiological fluids to assess local changes in specific body organs. Articles published in English between 1993 and 2019 were searched for in PubMed using terms DBP, vitamin D, and metabolites, inflammation. DBP is a transport protein and a regulator of immune and inflammatory processes.

Expert opinion: DBP capacity for transporting numerous ligands and co-involvement of DBP in immune and inflammatory processes suggest that DBP may be used in laboratory diagnostics as a specific parameter to confirm pathomechanisms of several systemic diseases and local conditions. Changes in the concentration of DBP present in a variety of clinical material may provide valuable information for use in assessing the severity and treatment of pathological processes.

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Vitamin D-binding protein; inflammation; neutrophil chemotaxis; acute phase proteins; vitamin D

1. Introduction

Vitamin D-binding protein (DBP) also known as Gc-globulin is a plasma glycoprotein with a molecular weight of 56–58 kDa composed of 458 amino acids and first identified by Hirschfeld in 1959. In humans, it is encoded by the *Gc* gene localized to chromosome 4 (4q11-q13) and its length is 1690 nucleotides. DBP is highly polymorphic with three commonly recognized variants (Gc1F, Gc1S, and Gc2) and over 120 rare variants. The DBP–encoding gene is part of a gene cluster (the albumin multigene family) that includes albumin, alpha-fetoprotein and afamin, exhibiting amino acid sequence homology. DBP is primarily synthesized by hepatic parenchymal cells and expressed in several tissues, including the liver, kidney, gonads, fat, and neutrophils. The DBP molecule contains two longer domains (I and II) and a shorter, C-terminal domain (III) which differ in specificity and localization of various ligand-binding sites [1–5].

This paper is a literature review focusing on the varied biological functions of DBP and its potential uses as a laboratory marker. Articles published in English between 1993 and 2019 were searched in PubMed using the terms DBP, vitamin D and metabolites, inflammation, neutrophil chemotaxis.

2. The biological roles of DBP

DBP performs a variety of functions reflecting its two main roles. Generally, it serves as a transporter for various ligands and it is involved in the regulation of the immune and inflammatory processes.

2.1. The transport function of DBP

DBP binds vitamin D and with different affinities vitamin D analogues at the binding site located at the N-terminal domain I and independently of that actin by the C-terminal domain III.

2.1.1. Transport of vitamin D and its metabolites

DBP is primarily the plasma carrier for vitamin D and its metabolites although its plasma molar concentrations exceeding by 50-fold those of its ligand vitamin D metabolites suggest that DBP also has other important physiological functions. 25(OH)D (25-hydroxyvitamin D) is the best-studied form of vitamin D as it provides the best measure of vitamin D status. In a normal non-pregnant individual approximately 0.03% of 25(OH)D is free, 85% is bound to DBP, 15% is bound to albumin [6,7] Binding affinity for DBP of $1,25(OH)_2D$ is lower than that of 25(OH)D. Due to the high binding capacity of DBP for vitamin D and its metabolites, DBP regulates their bioavailability by increasing their biological half-lives and protecting them from the hydroxylase-mediated catabolism. DBP also prevents an excess supply of free vitamin D to the tissues. DBP genotyping variability is a significant factor in determining vitamin D status associated with recommended vitamin D intake. Variations in DBP amino acid sequence appear to alter the binding affinity of vitamin D ligands for DBP [2,6,8–14].

2.1.1.1. Mechanism by which DBP interacts with target cells. Only the non-bound fraction (the free fraction) is able to enter cells and exert their biologic effect. It is a very small

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Article Highlights

- DBP has properties of both systemic laboratory parameter measured in the blood plasma and specific parameter measured in a variety of physiological fluids to assess local changes in specific body organs.
- DBP serves as a transporter for vitamin D and with different affinities vitamin D analogues, binds with high capacity actin and is a carrier of saturated and unsaturated free fatty acids.
- DBP is involved in immune and inflammatory processes through macrophage and osteoclast activation and via C5a-co-chemotactic activity.
- DBP is the most polymorphic protein known, and different DBP alleles can have a substantial impact on its biological functions.
- The variability of DBP concentrations in physiological fluids depends on Gc polymorphism, non-specific acute phase response, hormones which regulate DBP production and affinity of DBP to bind with multiple cell-surface ligands.
- DBP concentrations determined in various clinical materials (urine, cervicovaginal fluid, bronchoalveolar lavage, gingival crevicular fluid, fresh thrombotic cells, synovial fluid, sputum, cerebrospinal fluid) may provide useful information on the functioning of the specific organs.

percentage that is not protein bound (0.03%) in normal individuals that is able to cross the membrane of most cells. Although megalin/cubilin – as multi-ligand receptor acts as cell surface receptor for DBP in renal vitamin D capability most extra-renal tissues do not appear to express megalin or its associated co-receptors [6,7].

2.1.1.2. Estimations of free 25OHD. Assays to directly measure free 25(OH)D are not currently available for use in clinical care. Mathematical estimations of free 25OHD have relied on equations that utilize average binding coefficients for DBP and albumin. The calculation of free 25(OH)D depends on the concentrations of DBP. Bioavalilable 25(OH)D refers to all circulating 25(OH)D that is not bound to DBP, which is free plus that which is bound to albumin. It represents approximately 10% of all circulating 25(OH)D and has been used as an alternative to the free 25(OH)D in some clinical studies [6,7].

2.1.2. Actin scavenger

The binding capacity of DBP for actin is high. Actin is a component of the cytoskeleton involved in the cell motility and maintenance of cell shape. During normal cell turnover, tissue damage and cell death actin are released into the extracellular milieu. Release of actin complexes DBP from damaged cells is a common feature in all types of injury. The preference of DBP for binding actin is vital in preventing actin polymerization, which might result in the vasculature occlusion following cellular damage [2,3,14–19].

2.1.3. Transport of fatty acids

DBP is a carrier of saturated and unsaturated free fatty acids and may be involved in lipid metabolism. Mono- and polyunsaturated fatty acids with different specificities decrease the affinity for DBP of both 25(OH)D and 1,25(OH)2D. Fatty acid binding utilizes a single high-affinity site for both palmitic acid and arachidonic acid, but only arachidonic acid competes with 25(OH)D binding [6]. The capacity of binding plasma oleic acid which is a tonic inhibitor of neutrophil chemotaxis suggests DBP involvement in this process [8,15].

2.2. The anti-inflammatory and immunomodulatory function of DBP

2.2.1. Macrophage and osteoclast activation

DBP is converted into a macrophage–activating factor (DBP-MAF) by the action of either beta-galactosidase from beta lymphocytes or sialidase from T lymphocytes on the carbohydrate side chains of the protein. Deglycosylated DBP may affect phagocytic and tumoricidal macrophage activity and osteoclast differentiation, control the immune response and induce apoptosis in the activated macrophages. Since macrophages perform a key role in cytokine production during the inflammatory response, DBP is likely to act as a mediator of this process. DBP-MAF is an antiangiogenic molecule that can act directly on endothelium as well as stimulate macrophages to attack both the endothelial and tumor cell compartment of a growing malignancy [2,3,8]. The Gc2 isoform is less readily converted into the deglycosylated form (DBP-MAF) compared to the Gc1f and Gc1s.

2.2.2. C5a-co-chemotactic activity

DBP augments monocyte and neutrophil chemotaxis to inflammatory foci via the activation of the C5a-mediated signaling. The interaction between DBP and cell-surface receptors is the underlying mechanism of increases in the C5a-dependent recruitment of immunocompetent cells [2,3,8,10,18].

3. The effects of modulatory factors on the variability of DBP concentrations in physiological fluids

3.1. Gc polymorphism

The total circulating levels of DBP and its biological functions may depend on the specific Gc phenotype. Over 120 variants have been described, of these Gc1f, Gc1s and Gc2 are the most common. Phenotypic variations in DBP are polymorphism in the gene for DBP. The composite genotype of two single nucleotide polymorphisms (rs7041 and rs4588) results in different DBP isotypes. DBP alleles have been linked to serum levels of DBP with Gc2 being the least abundant and Gc1f the most abundant. The main phenotypic variants of DBP are known: Gc1-1, Gc1-2, and Gc2-2, of which Gc1-1 has the highest plasma concentration (Gc1s-1s, Gc1s-1f, Gc1f-1f), lower concentrations have been shown for Gc1- 2 (Gc1s-2 and Gc1f-2) and the smallest for Gc2-2. The protein configurational differences among DBP isotypes affect DBP substrate-binding affinity and can have a substantial impact on its biological functions. Recent publications have shown a correlation between serum concentrations of 25(OH)D and DBP. The differences in the capacity of DBP variants for binding vitamin D in domain I may have an impact on serum 25-OH-D levels and vitamin D availability to cells. The proven association between the genetic variants of DBP and 25(OH)D concentration demands individual assessment of these two parameters when vitamin D supplementation is considered. The three most common alleles – Gc1f, Gc1s, Gc2 – differ in their affinity with vitamin D metabolites and have been variably associated with a number of clinical conditions – Gc1f have the highest avidity for vitamin D metabolites and Gc2 the lowest.

A considerable DBP polymorphism has been described with a specific allele distribution in different geographic areas. Gc alleles show distinct racial distribution patterns: Gc2 is the most common isoform within the white race and Gc1f within the black race.

Many studies suggest an association between DBP phenotypes and resistance or susceptibility to thyroid diseases, diabetes, osteoporosis, chronic obstructive pulmonary disease (COPD) or the occurrence of spontaneous preterm labor. Deglycosylated form DBP (DBP-MAF) plays an important role in macrophage activation and differentiation, which may support the putative link between DBP polymorphism and increased susceptibility to the development of certain diseases. It is thought that the Gc2 isoform is less readily converted into the deglycosylated form (DBP-MAF) since it possesses only one glycosylation site compared to the Gc1F and Gc1S which possess two independent O-glycosylation sites [1,3,5,8–10,20–27].

3.2. Acute phase response

DBP is implied to be an acute phase reactant with nonspecific immune defense and inflammatory functions. Clinical studies confirmed the characteristic properties of DBP as a positive acute phase protein whose synthesis in the liver could be upregulated by pro-inflammatory cytokines such as IL-6. When tissue death occurs, DBP levels significantly decrease due to DBP capacity for forming complexes with monomeric G-actin released from damaged muscle tissue. The process by which the DBP-G-actin complexes are removed from the circulation by the reticuloendothelial system increases the consumption of DBP and lowers its concentrations much more efficiently than it happens in the case of free DBP. Experimental studies have shown that the *in vivo* half-life for the DBP-G-actin complex is approximately 30 min compared to 24 h for actin-free DBP [3,4,23].

3.3. Hormones

DBP production may be regulated by estrogens, insulin, estradiol, triamcinolone, dihydrotestosterone.

3.3.1. Estrogens

Estrogens have an impact on increases in DBP synthesis or decreases in its catabolism and are a major cause of significantly higher DBP levels in women compared to men and of substantial DBP elevations during pregnancy and in women using hormonal contraception. Exact mechanism for this induction is not clear. A negative correlation between DBP levels and age in women suggests that age may be an independent factor affecting DBP levels. This is confirmed by lower DBP levels in post-menopausal women compared to pre-menopausal women. Increased estrogen levels may influence the total plasma concentrations of vitamin D metabolites. The use of hormonal contraception is associated with higher concentrations by 13–25% of the total vitamin D metabolites and DBP. Dynamic changes of DBP concentrations in women in different age groups, during pregnancy and in hormonal contraception users indicate the factors that should be taken into account in clinical practice when looking for causes of altered serum 25(OH)D levels and considering vitamin D supplementation.

3.3.2. Insulin

A negative correlation between insulin and DBP levels was demonstrated in obese adults who were found to have lower DBP levels than normal weight subjects which suggest that insulin may inhibit DBP production [6,9,25,26].

3.4. DBP binding to cell surface

DBP appears to bind with low affinity to multiple cell-surface ligands, such as chondroitin sulfate proteoglycans, megalin or cubulin. The fact that DBP can be readily bound to and internalized by the target tissue cells may indicate its active, specific and biologically important roles in the human body. The megalin and cubulin receptors in the proximal renal tubules bind and internalize DBP via an endocytic pathway. Although the mechanism of vitamin D uptake by endocytosis was first identified in the renal cells, it also occurs in other cells, including macrophages. Binding of DBP to neutrophil surfaces is considered to be necessary for the co-chemotactic activity of DBP, and these binding sites are reported to be up-regulated when neutrophils are activated by lipopolysaccharides. Circulating leukocytes probably possess a high-affinity binding site for native DBP and thus may contribute to systemic changes in DBP levels and rapidly deplete plasma DBP pool during circulation [3,11,22,24,25].

4. The pathomechanism of changes in DBP plasma concentrations

4.1. The reference values of DBP plasma concentrations

In the study by Lauridsen *et al.* the mean Gc concentration varied from 226 mg/L for subjects with the Gc2-2 phenotype to 274 mg/L for those with the Gc1S phenotype. No statistically significant difference was observed for individuals bearing one or the other of the Gc1S or Gc1F types, whereas a strong significant difference was observed for Gc2-2, Gc2-1, and Gc1-1. The genetic polymorphism was considered to be associated with race and ethnicity [20].

Different authors have reported various circulating plasma DBP concentrations in healthy individuals: 6 to 7 µmol/L [28], 77.51–175.1 µg/mL [23], 200 to 600 mg/l [10], 350 to 550 mg/L (6.25–9.8 µmol/L) [14]. The median level was found to be significantly higher in woman than in men. The diagnostic value of DBP measurements in organ damage may be enhanced by measuring both actin-free and actin-bound DBP [23,28,29].

Accurate measurements of DBP are needed to promote understanding of the relationship between DBP and 25(OH)D concentrations and to elucidate the role of DBP in health and disease. According to literature data quantification of DBP is typically performed using antibodies, but also as a tool was evaluated for quantification of total DBP and the three common isoforms liquid chromatography coupled with isotope dilution mass spectrometry (LC-IDMS) [30].

Study compare measures of DBP using a monoclonal versus polyclonal ELISA yielded highly discrepant measures of DBP particularly among black individuals, likely related to established race differences in DBP polymorphism. Serum DBP

concentrations varied widely and were inversely associated with black race [31].

4.2. Diagnostic significance of elevated plasma DBP concentrations

Elevated plasma DBP concentrations may be due to the following factors:

- *High estrogen concentrations*. The physiological and pathological conditions associated with high estrogen concentrations include pregnancy and hormonal contraception use. Elevated levels of DBP, especially the DBP-actin complex in maternal serum during pregnancy may result from the high turnover of trophoblasts in the placental villous tissue that is in direct contact with maternal blood [1,32].
- *Acute phase response*. Elevated plasma concentrations of DBP were associated with disease severity and positively correlated with neutrophil counts and neutrophil percentages as well as with increased levels of systemic inflammatory markers like C-reactive protein, IL-6 and procalcitonin [3,20,23,33].

4.3. Diagnostic significance of reduced plasma DBP concentrations

4.3.1. Assessment of the extent and dynamics of destructive changes in the course of organ dysfunction in critically ill and injured patients

In acute tissue damage, plasma DBP concentrations decrease which is accompanied by increases in the DBP-actin complex formation. Of importance for clinical practice is the difference in the diagnostic interpretation between the two parameters, namely actin-free DBP and actin-bound DBP. A homeostatic mechanism known as the actin scavenging system becomes active soon after acute injury and is responsible for reduced plasma concentrations of DBP due to its increased consumption. Considering that the half-life for actin-free DBP ranges from 12 to 24 h while that for the DBP-actin complexes is approximately 30 min, increases in DBP complexed with actin may lead to a more rapid total DBP turnover. Differentiating between the diagnostic significance of actin-free and actin-bound DBP would increase their value as prognostic markers for early identification of an increased risk of lethality in severe organ damage such as acute or fulminant hepatic failure, paracetamol overdose, multiple trauma, and multiple organ failure. In patients with fulminant hepatic failure low DBP levels may be a predictor of multiple organ failure. Selective detection of actin-free DBP in human serum allows the monitoring of trace amounts of DBP reserve in critically ill patients. Rapid decreases in the circulating actin-free DBP levels may be indicative of developing disseminated intravascular coagulation. Prospective analysis of changes in plasma actin-free DBP concentrations may aid therapeutic decision-making in trauma patients with constant increases in actin release as well as serve as a predictor of survival after severe injuries. In acute illness due to actin binding and accelerated clearance form circulation changes in DBP correlated with changes in 25(OH)D [3,10,14,16,23,34–38].

4.3.2. Chronic disease

The mechanism underlying decreases in plasma DBP concentrations observed in chronic disease differs from that observed in acute conditions.

4.3.2.1. Chronic liver disease, including chronic and acute-on-chronic liver failure and chronic cirrhosis. In these conditions, plasma concentrations of actin-free DBP were significantly lower compared to healthy controls. Considering that DBP is synthesized in the liver, in hepatic insufficiency serum DBP concentrations are decreased and in consequence the condition further aggravated by interference with the actin-scavenging system and DBP function as the precursor protein for the macrophage-activating factor. Decreased DBP production may be critical in chronic liver disease. In profound liver dysfunction, low DBP levels probably have an impact on low levels of total vitamin D [27,39].

4.3.2.2. Diabetes mellitus. Decreased serum DBP concentrations and insufficient levels of vitamin D may increase the risk of type 1diabetes development. This is important for the identification of women with low DBP levels during pregnancy whose children would develop type 1diabetes later on in life. It has been suggested that vitamin D may have a direct effect on beta-cells, including improving insulin secretion, enhancing expression of the vitamin D receptor and improving islet morphology. The DBP gene polymorphism is likely to influence vitamin D levels predisposing to gestational diabetes mellitus (GDM). Administration of vitamin D reduces abnormal glucose tolerance in pregnant women with GDM by altering insulin sensitivity. Urinary loss of DBP may be another factor responsible for decreased serum 25(OH)D levels during pregnancy [25,26,40,41].

4.3.2.3. Inflammatory response. Decreases in plasma DBP levels may be the effect of DBP conversion into the vitamin D-binding protein-macrophage activating factor (DBP-MAF) with the involvement of B- and T-lymphocytes. Both human B-lymphoid cells and activated normal B- and T-lymphocytes take up DBP. It has been suggested that this endocytosis facilitates intracellular delivery of vitamin D, which supports the hypothesis that the delivery of vitamin D metabolites plays a role in the physiological response to injury [2,3,8].

4.3.2.4. Effect of receptor-mediated DBP binding to cell-surface receptors. Plasma DBP binds to the surface of several cell types, including neutrophils. Since the amount of cell-bound DBP is unknown, a question arises to what extent the actual numbers and activation of the circulating neutrophils influence the variations in plasma DBP concentrations [2,18].

5. Pathophysiological role of the cell surface DBP binding sites

There is a growing body of evidence confirming the hypothesis that DBP binding to the surface of several cell types is the key factor regulating DBP functions. The authors report the

observation of cell-associated DBP. Studies using immunofluorescent or radioisotope techniques demonstrated DBP binding to the plasma membrane of human blood monocytes and lymphocytes, human placental trophoblast and smooth muscle cells, rat pancreatic acinar cells, and porcine kidney tubule cells. A number of molecules have been identified as potential DBP binding sites, including chondroitin sulfate proteoglycan, CD44, annexin 2, megalin, and cubulin on proximal tubule cells in the kidney, and actin. Different cell types are likely to have different cell surface ligands for DBP.

Binding to the cell surface is essential for DBP to function as [2,4,27,42,43]:

- a chemotactic cofactor for C5
- a macrophage or osteoclast activating factor
- an effector of DBP-actin complex clearance by the liver
- a carrier of vitamin D sterols and free fatty acids to cells

5.1. The effect of DBP on the regulation of vitamin D transport to cells

More than 90% of 25(OH)D circulating in the human serum is DBP-bound and as a result any changes in the concentrations of this carrier protein affect the total concentrations of vitamin D and its metabolites. Numerous published studies indicate the role DBP plays in vital metabolic processes involving 25 (OH)D delivery to cells. This poses the question whether DBP-mediated delivery of vitamin D metabolites to the target cells may depend on the regulation of the receptor-mediated membrane transfer and the organ-specific demand of cells for vitamin D. There is evidence that the DBP gene polymorphisms associated with insufficient vitamin D amounts delivered to specific organs may be responsible for the development of type 1 diabetes and GDM, predisposition to type 2 diabetes, autoimmune thyroid disorders and impaired lung function in COPD. Knowledge about the possible effect of DBP on the optimal immune response could explain the affinity differences and correlations between the DBP gene polymorphisms and thyroid disorders, diabetes, obesity, and other diseases. Considering the impact of various physiological and pathological factors as potential confounders making the precise interpretation of plasma total 25(OH)D concentrations difficult, the clarification of DBP role in these pathological processes is of vital importance [5,11,13,25,35,43].

5.2. Neutrophil-associated DBP and local inflammation

Normal human neutrophils contain DBP on the cell surface and in the intracellular pool. The most likely source for neutrophil-associated DBP is either the preexisting pool generated in the bone marrow and/or acquisition of DBP by endocytosis while the circulating neutrophils are bathed in plasma. Considering the massive number of neutrophils that can migrate to a site of inflammation, DBP potentially could be released at the inflammatory site.

Neutrophil-associated DBP could have numerous physiologic roles in local immune and inflammatory responses, including:

(1) binding and sequestration of microbial actin released during phagocytosis.
(2) autoregulation of actin polymerization during chemotaxis and phagocytosis
(3) binding and intracellular transport of vitamin D sterols
(4) regulation of complement-derived chemotactic activity [28].

5.2.1. DBP and other regulators of chemotactic activity for neutrophils at local sites of inflammation [null–null]

DBP can bind C5a and its metabolite C5a des-Arg and enhance their chemotactic activity for neutrophils. This complex is functionally independent of C5a binding to the C5a receptor since DBP does not alter the C5a receptor–ligand interactions or bind to C5a or the C5a receptor. The chemotactic cofactor functions of DBP at local sites of inflammation require the chemotactic signal, neutrophil activation, binding of DBP to the cell surface and DBP shedding into the extracellular milieu. Plasma membrane binding and subsequent shedding of DBP are the critical steps required for this protein to perform its biological functions.

The role of the membrane binding side: Activated neutrophils bind DBP to the plasma membrane binding sites such as CD44, annexin 2, actin and display enhanced chemotaxis to C5a.

Shedding of DBP from the plasma membrane: The release of soluble DBP forms into the extracellular milieu is controlled by the proteolytic process in which neutrophil elastase, a serine protease cleaves the DBP binding site (but not DBP) and sheds it into the extracellular milieu. Treatment of neutrophils with elastase inhibitors allows DBP to accumulate on the cell surface.

Inhibition of DBP shedding: The inhibition of elastase activity by its specific inhibitors and a decrease in temperature below 37° are the main stimuli involved in the termination of the DBP binding/signaling.

5.2.1.1. DBP may neutralize an endogenous inhibitor of chemotaxis. Plasma oleic acid is a tonic inhibitor of neutrophil chemotaxis. DBP binds oleic acid to scavenge inhibitory fatty acids in physiological fluids.

6. DBP concentrations determined in various physiological fluids as a diagnostic tool

DBP occurs at high concentrations in plasma and a number of other physiological fluids, including breast milk, seminal fluid, cerebrospinal fluid, saliva, and bronchoalveolar lavage fluid. The finding of the protein in various clinical specimens of body fluids may provide clinically useful information on the functioning of the relevant organs [3,10,44].

6.1. DBP in urine

Urinary DBP (uDBP) may serve as a diagnostic marker for three conditions.

6.1.1. Proteinuria severity

The molecular weight of DBP (56–58 kDa) is in the upper limit on the molecular weight of deliverable molecules which allows DBP filtration at the glomerulus and subsequent reabsorption by proximal tubular cells mediated by two endocytic receptors, megalin, and cubulin. In the course of chronic kidney disease, increases in uDBP correlate negatively with the creatinine clearance and positively with proteinuria. This increase associated with the severity of diabetic nephropathy confirms that uDBP is a potential biomarker for early diabetic nephropathy [8,12,25,43].

6.1.2. Renal interstitial inflammation and fibrosis

uDBP is strongly associated with the markers of both early and late tubulointerstitial fibrosis (alpha-SMA and collagen III, respectively) even independent of albuminuria. Increases in uDBP have been observed in tubular dysfunction (nephrotic syndrome and renal Fanconi syndrome). It has been suggested that uDBP may be used as a noninvasive marker in monitoring of the severity of tubulointerstitial inflammation and fibrosis [12,45]

1,25(OH)$_2$D regulation. 25(OH)D is specifically targeted to the proximal tubule through receptor-mediated uptake of its carrier protein DBP. This process is crucial for the retrieval of vitamin D for activation by 1-alpha hydroxylase, which is abundantly present in proximal tubular cells, for subsequent intracellular conversion of 25(OH)D into 1,25(OH)$_2$D. An increase in the urinary loss of DBP may lead to decreases in total vitamin D levels in patients with proteinuria [13,25].

6.2. DBP in cervicovaginal fluid (CVF)

The presence of DBP in the CFV during pregnancy is the result of plasma transudate. Higher concentration of DBP in the CVF may be indicative of increased inflammation and tissue damage, which are integral to cervical remodeling and fetal membrane weakening with approaching labor onset [1,46,47].

6.3. DBP in bronchoalveolar lavage fluid (BALF)

The diagnostic role of DBP present in BALF may be associated with different biological functions of this protein [23,33].

6.3.1. Enhancing monocyte responses to c5-derived peptides

Increased lymphocyte counts in the BALF from patients with lung diseases such as COPD and allergic asthma are a potential source of enzymes released by lymphocytes and DBP conversion into the macrophage-activating factor (DBP-MAF). Hence, the need to control the balance between the beneficial anti-inflammatory and immunomodulatory effect of DBP resulting the increased accumulation and activation of macrophages in the lung tissue and the potential damage to the lung tissue by macrophages which significantly contributes to the development of COPD [5,23].

6.3.2. Effect on neutrophil chemotaxis and actin binding

DBP in BALF may be derived from plasma leakage or reach the lung tissue by other mechanisms. A high correlation between DBP and neutrophils in BALF in allergic asthma indicates that DBP contributes to neutrophil recruitment into the lung. Significant increases in DBP and 25(OH)D in human BALF in the asthmatic late-phase reaction 24 h after the allergen challenge may result from their release from neutrophils into the extracellular space effected by serine protease activity [45].

6.4. DBP in gingival crevicular fluid

Patients with generalized aggressive periodontitis had higher plasma concentrations of DBP but lower gingival crevicular fluid levels of the protein than healthy controls. Decreased DBP levels in the gingival crevicular fluid of these patients may be associated with DBP binding to the surface of inflammatory cells as well as fibroblasts and smooth muscle cells [23].

6.5. DBP in fresh thrombotic cells

High expression of DBP in fresh thrombotic plaques and in the sera of patients with ST-elevation myocardial infarction supports the role of plasma DBP as a biomarker for vascular injury with significant prognostic and diagnostic implications [38].

6.6. DBP in synovial fluid

Synovial fluid DBP correlates inversely with swollen joint and have the potential to influence rheumatoid arthritis severity and progression [48].

6.7. DBP in sputum

DBP in sputum show higher abundance in active tuberculosis and remain unchanged in systemic circulation which suggests altered patho-physiological system in lungs of tuberculosis patient [49].

6.8. DBP in cerebrospinal fluid

DBP may be a potential useful biomarker for diagnosis or medicine target for treatment of multiple sclerosis. The levels of DBP in cerebrospinal fluid may vary according to the stage (acute or chronic), duration and severity of the disease. There is an association between down-regulated DBP levels and multiple sclerosis [50].

7. Conclusions

DBP has properties of both systemic laboratory parameter measured in the blood plasma and specific parameter measured in a variety of physiological fluids to assess local changes in specific body organs. The variability of DBP concentrations in physiological fluids depends on Gc polymorphism, nonspecific acute phase response, hormones which regulate DBP production and affinity of DBP to bind with multiple cell-surface ligands. DBP concentrations determined in various clinical materials (urine, cervicovaginal fluid, bronchoalveolar lavage, gingival crevicular fluid, fresh thrombotic cells, synovial fluid, sputum, cerebrospinal fluid) may provide useful information on the functioning of the specific organs.

8. Expert opinion

DBP is one of the most abundant serum proteins and transports 85–90% circulating vitamin D metabolites. 25(OH)D (25-hydroxyvitamin D) is the best-studied form of vitamin D as it provides the best measure of vitamin D status. In a normal non-pregnant individual approximately 0.03% of 25(OH)D is free, 85% is bound to DBP, 15% is bound to albumin. Binding affinity for DBP of $1,25(OH)_2D$ is lower than that of 25(OH)D. Only the non-bound fraction (the free fraction) is able to cross the membrane of most cells and exert their biologic effect. Although megalin/cubilin – as multi-ligand receptor acts as cell surface receptor for DBP in renal vitamin D capability, most extra-renal tissues do not appear to express megalin or its associated co-receptors. Due to the high binding capacity of DBP for vitamin D and its metabolites, DBP regulates their bioavailability by increasing their biological half-lives and protecting them from the hydroxylase-mediated catabolism. DBP also prevents an excess supply of free vitamin D to the tissues. It is the product of one of the most polymorphic genes in the human population. DPB variants may directly impact the interpretation of clinical data as the isoforms have different binding affinities for vitamin D metabolites and are associated with discriminatory serum concentrations of these metabolites. DBP is not only a serum protein that transports vitamin D, but it also attaches to other ligands such as actin and free fatty acids, indicating that these compounds may be co-involved in the regulation of certain metabolic processes. DBP is involved in immune and inflammatory processes through macrophage and osteoclast activation and via C5a-co-chemotactic activity. The variability of DBP concentrations in physiological fluids depends on Gc polymorphism, nonspecific acute phase response, hormones which regulate DBP production and affinity of DBP to bind with multiple cell-surface ligands. The role of DBP in the regulation of immune and inflammatory processes and the presence of DBP in a variety of clinical material (serum, urine, cervicovaginal fluid, bronchoalveolar lavage fluid, gingival crevicular fluid, fresh thrombotic cells, synovial fluid, sputum, cerebrospinal fluid) may provide specific diagnostic information on the activity of local disease conditions in such organs as the kidney and lung or in vascular endothelium. The literature offers information on astonishingly diverse DBP involvement in many physiological and pathological processes, both acute and chronic. The authors present DBP as a valuable marker for a wide range of diseases, including liver disease, sepsis, tuberculosis, cystic fibrosis, type 1 diabetes, ST-elevation myocardial infarction, osteoporosis, thyroiditis, chronic obstructive pulmonary disease, acquired immune deficiency syndrome (AIDS), multiple sclerosis, sarcoidosis, rheumatoid fever, renal interstitial inflammation and fibrosis . Despite this, DBP is not a commonly used laboratory marker. The knowledge about the pathomechanisms of this protein involvement in numerous metabolic processes presented in this paper justifies its use as a biomarker to confirm specific clinical diagnoses suggested by nonspecific signs and symptoms. Further studies combined with the validation and standardization of DBP assays in specific clinical material may provide valuable information for use in assessing the severity and treatment of conditions associated with changes in the concentration of DBP.

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Declaration of interest

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