Medical and Research Publications
International Open Access

Review Article **Journal of MAR Case Reports (Volume 6 Issue 3)**

The Feasibility of Potential Utilization of Bilirubin as a Biomarker &Therapeutic Target for Treatment of Diabetic Kidney Disease: A Narrative Review

Dr Kulvinder Kochar Kaur,M.D *, Dr Gautam Nand K Allahbadia M.D[1], Dr Mandeep Singh M.D.DM[2]

1. (Obstt & Gynae), D.N.B, Scientific Director, Ex-Rotunda-A Centre for Human Reproduction 672, Kalpak Garden, Perry Cross Road, Near Otter's Club, Bandra(W)-400040 MUMBAI, INDIA
2. (Std) (Neurology) Consultant Neurologist Swami Satyanand Hospital Near Nawi Kachehri, Baradri, Ladowali road, JALANDHAR, PUNJAB.

Corresponding Author: Dr Kulvinder Kochar Kaur, M.D, (Obstt & Gynae, specialist reproductive endocrinology & Infertility specialist). Centre For Human Reproduction Scientific Director cum Owner 721,G.T.B. Nagar, Jalandhar-144001, Punjab, India

Received Date: November 14, 2022

Published Date: December 01, 2022

Abstract

*Bilirubin has been believed to be a robust endogenous antioxidant along with **a**nti-inflammatory molecule possessing the capacity of working on cellular pathway in the form of a hormone. Diabetic Kidney Disease (DKD), is in general a usually encountered complication, reflecting a frequent etiology of end stage renal Disease(ESRD) as reviewed by us earlier regarding different etiologies of DKD and avoidance of its propagation. The group of Vitek, Bianco, Bellarosa C, Tiribelli C, networking in last decade have recently highlighted the part of bilirubin as a potential therapeutic substancefor DKD. Thus here we provide a narrative review regarding the molecular along with clinical properties of mild hyperbilirubinemia in the form of this additional mode for treating DKD.As earlier reviewed the pathogenesis implicated in the generation of DKD are inclusive of Oxidative stress(OS), apoptosis, inflammation in addition to fibrosis. There is a positive correlation amongst serum bilirubin quantities with the quantities of antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase(GPx), that are inversely associated with the quantities of C Reactive Protein(CRP), tumor necrosis factor α (TNF-α), interleukin-6(IL-6) , IL-12, IL-10, in case of DKD. Bilirubin results in downregulation of nicotinamide adenine dinucleotide phosphate reduced form(NADPH), along with reduction of proapoptotic Hypoxia inducible factor 1 (HIF 1α), cleaved caspase3, as well as cleaved poly ADP ribose polymerase(PARP) induction that illustrated lesser DNA fragmentation. More recently, experimental along with clinical studies have illustrated its actions on the generation as well as propagation of the renal diseases, that suggest that just mild escalation of serum bilirubin quantities cause actual clinical advantages. Nevertheless, there exist requirement for future randomized controlled studies for the assessment of the exact part of bilirubin quantities in the form of a marker or as a therapeutic target for DKD is actually possible.*

Key Words: *Bilirubin; DKD; Oxidative stress (OS; apoptosis; inflammation; fibrosis; antioxidant; **a**nti-inflammatory.*

Introduction

Regarding historic facts bilirubin was believed to be a nonfunctional heme catabolism waste product as well as a sign of liver disease or in maximum inimical scenario probably a neurotoxic molecule. In view of mammalian utilization of energy along with resources transforming biliverdin(ie a non-toxic substance which can be liberated by ease) into bilirubin(which requires to get metabolized to get excreted via the biliary system)it can be assumed that bilirubin is more than simply a breakdown end product of heme catabolism .Over time gradually in past few 20-30 years it got appreciated by the Clinicians that mild hyperbilirubinemia was manifested by Gilbert's patients(however quantities in the upper quartile of the presently agreeable physiological serum bilirubin range) conferred protection against diseases believed to be a result of modern civilization (like diabetes mellitus(DM), cardiovascular disease (CVD) , Metabolic Syndrome(MetS)) diseases, dependent basically on Oxidative stress(OS).In the meantime researchers gradually initiated the evaluation of mode behind the protection offered by this molecule, along with in combination with its antioxidant characteristics in the form of a Reactive oxygen species(ROS)forager , they made the invention that biliary works in the form of a considerably significant modulator of biological function in the human body besides working in the form of a hormone , directly targeting its receptor**[**1**]**.In view of minimal escalation of serum bilirubin quantities apparently are significant in resulting in reduction of OS- dependent diseases, researchers are taking into account the probability of enhancing serum bilirubin quantities in the form of an avoidance approach against these modern so called civilization diseases**[**2**]**.

Diabetic Kidney Disease (DKD) takes place in about 20-40% of patients presenting with type1 or type2 diabetes mellitus (DM). Early diagnosis of DKD is cardinal for avoidance of propagation towards end stage renal disease (ESRD). Present research is concentrated on looking for strategies regarding anticipation of avoidance of DKD propagation along with enhancing the treatment **[**3**]**.

Endogenous bilirubin apparently was key both in the form of probability of a marker regarding propagation along with in the form of a therapeutic target for the avoidance of propagation of DKD. As per the various recent studies, total serum bilirubin quantities could be believed to be a marker of DKD propagation aiding in picking up low as well as high risk patients group. Patients possessing the low normal total bilirubin quantities might get greater combative management for postponement of propagation to Kidney failure. In the form of a marker bilirubin possesses the benefit of getting estimated with ease, economical along with in the form of a routine investigation in maximum centres. Greater studies are needed for estimating if the total bilirubin quantities in the form of a therapeutic target with the idea of avoidance of Chronic Kidney Disease (CKD)works. Numerous measures

possess the capacity of mild escalation of serum bilirubin quantities inclusive of lifestyle initiation alterations, utilization of natural substances in the form of nutraceuticals or chemical drugs besides probability of utilization of bilirubin in nanoparticles **[**4**]**.

Bilirubin

Bilirubin metabolism

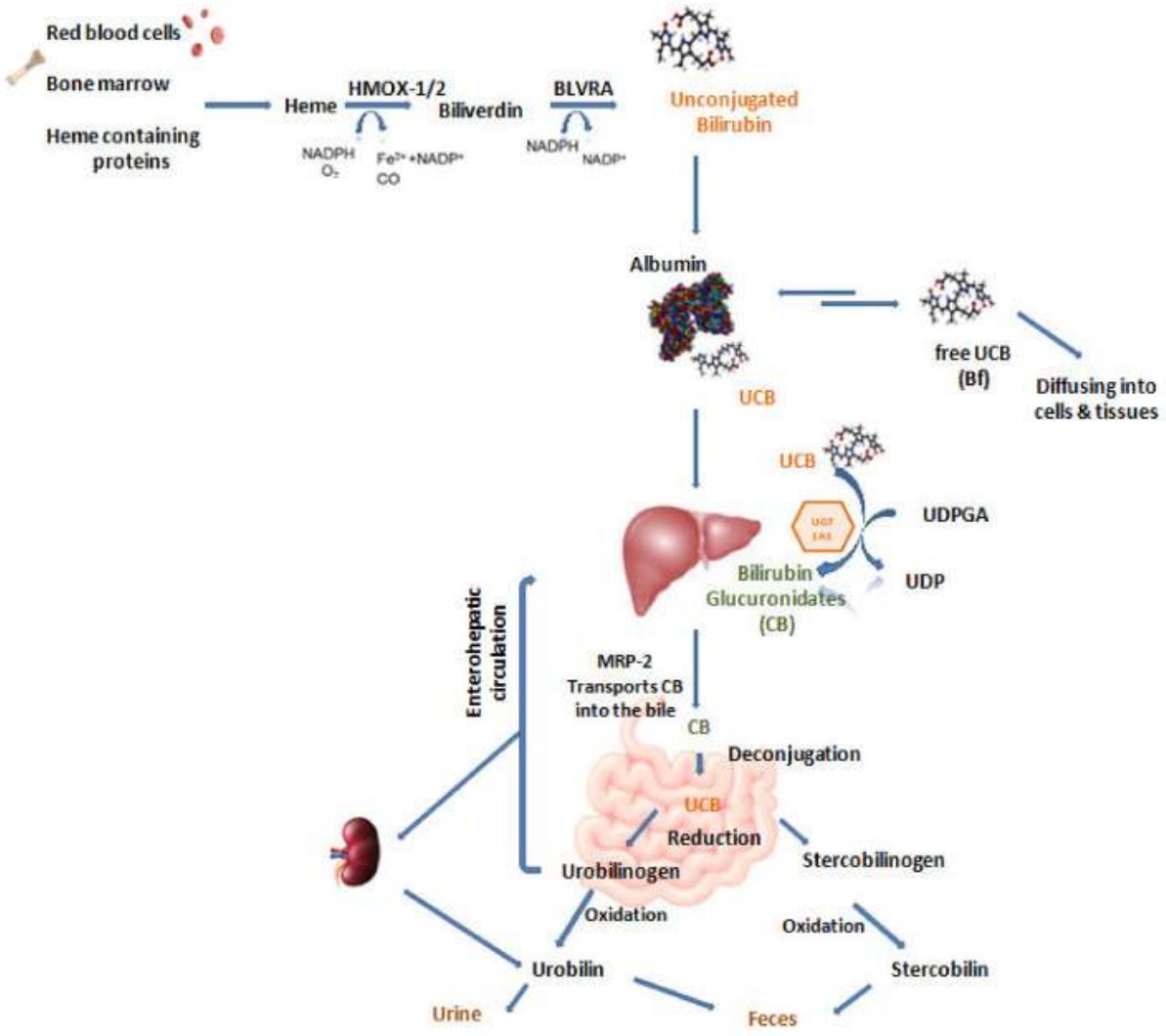

Figure 1 Courtesy ref no-9-Bilirubin metabolism. HMOX-1/2: hemeoxygenase enzyme; NADPH: nicotinamide adenine dinucleotide phosphate reduced form; O2: oxygen; Fe2+: ferrous ions; NADP+: nicotinamide adenine dinucleotide phosphate oxidized form; CO: carbon monoxide; BLVRA: biliverdin reductase A enzyme; UCB: unconjugated bilirubin; Bf: free bilirubin; UGT1A1: uridine diphosphate-glucuronosyl transferase 1A1; UDPGA: uridine 5′-diphosphoglucuronic acid; UDP: uridine diphosphate; CB: conjugated bilirubin;MRP-2:multidrug resistance-associated protein 2.

Bilirubin represents a tetrapyrrolic compound super family along with is the end product of heme catabolism whose production takes place basically in the splenic reticulo endothelial system [5].In a healthy adult generation of about 4.4± 0.7mg/kg by body weight of Bilirubin /day occurs[6].the erythrocytes that are senescent represent the major sources of the heme group, however not just the only source despite about 80% of this amounts get obtained from hemoglobin of senescent red blood cells (RBCS), the rest part get shared amongst the turnover of myoglobin, (another protein possessing the heme group along with specialization in oxygen transportation), cytochrome c as well as other hemoproteins like microsomal cytochrome CYP-450[7].In toto provision of15-20%of the accessible substrates from these proteins in the end the breakdown of immature RBCs in the bone marrow donates the 3% of the heme group[8].

Bilirubin metabolism comprises of numerous steps inclusive of generation, hepatocyte uptake, conjugation, excretion into the bile ducts, besides delivery to the intestine(fig1) [rev in ref no-9]. Jaundice might develop from aberrations in either of the steps of bilirubin metabolism.

Initiation starts with the transformation by heme oxygenase1 enzyme (HMOX, Online Mendelian Inheritance of Man [OMIM1No*.131250) into biliverdin liberating CO, Fe2+, H2Oalong with current oxidating nicotinamide adenine dinucleotide phosphate reduced form (NADPH)-to NADP [nicotinamide adenine dinucleotide phosphate oxidized form].This enzyme results in induction of the opening of the heme ring, thus freeing the iron besides generation of the tetrapyrrolic chain . Subsequently reduction of biliverdin to bilirubin takes place by cytosolic biliverdin reductase [BLVRA, OMIM No*.109750]in the presence of NADPH [5].

In view of bilirubin being markedly insoluble in water (the solubility threshold in plasma being very low 70nm0l/L=0-04 mg/dl)[10]it can be brought about in the vascular bed by its binding to albumin, the protein present in maximum quantities in blood. In view of its robust binding capacity (Ka=7x107/M) in addition to greater quantities of serum albumin in human serum, maximum bilirubin is bound to albumin with only under 0.1% of bilirubin continues to remain unbound, hence known as free bilirubin [Bf]. The pathophysiological characteristics of this pigment are associated with the fraction of free bilirubin instead of total bilirubin)[11]. In lieu of albumin binding bilirubin possessing the capacity of gaining entry to the liver where the transportation of this pigment takes place actively inside the hepatocytes at the sinusoidal membrane through organic anion transporting poly Peptide1B1(OAT1B1) [OMIM1No*.604843)[6]. On reaching the hepatocytes cytoplasm solubilization of bilirubin takes place by particular binding proteins out of which the 2 maximum intriguing proteins are protein Y(ligandin α isoform of glutathione S transferase B) as well as protein

Z(liver particular fatty acid binding protein, FABP1)that are guided towards the endoplasmic reticulum (ER) 8**]**. Furthermore, for transport conjugation of bilirubin takes place in the liver with a molecule or two of glucuronic acid by the UDPglucuronosyl transferase 1 A1(UGT1A1) enzyme**[**12**]**.This conjugated bilirubin(CB)gets effectively liberated maximum by the ATP based MRP2(multidrug resistance associated protein) /ABCC2 transporter OMIM1No*.601107), along with gets thrown in the duodenum **[**13**]**. Amongst the intestine, practically complete bilirubin gets deconjugated. Maximum of that gets excreted in the form of urobilinoids **[**14**]**, whereas the rest of part gets excreted in the form of unconjugated bilirubin (UCB).Initially CB gets deconjugated into UCB by beta glucuronidase, then subsequently microbiota result in reduction to urobilinogen along with stercobilinogen. Stercobilin along with urobilin get excreted through faeces. Physiologically, a tiny part of UCB along with urobilinogen get absorbed via enterohepatic circulation. Urobilinogen shift to kidney for filtration followed by excretion via the urine **[**7**]**. UCB gets reabsorbed in the colon and gets shifted back to the liver via the portal circulation **[**15**]**.

Abnormalities in the UCB hepatic uptake as well as conjugation enhances serum UCB quantities, with a subsequent escalation of blood Bf fraction quantities**[**16**]**.Earlier we had reviewed the various modalities of treatment of Diabetic Kidney Disease(DKD)which represents the greatest stimulator of Chronic Kidney Disease(CKD) by trying Epigenetics modifications, besides that of acute kidney injury(AKI)with use of N-acetylcysteine & vitamin c for managing oxidative stress, role of Vitamin K deficiency, Vitamin D supplementation besides other specific etiologies of CKD**[**17-22**]**.Here we further provide a narrative review regarding part of bilirubin for avoidance along with treatment of DKD.

Bilirubin in the form of Janus Bifrons

The behavior of bilirubin group in human body possesses 2 faces, akin to Janus Bifrons as good as God. Robust hyperbilirubinemia that lasts for a considerable duration result in neuronal damage or bilirubin encephalopathy (alias kernicterus). The properties of kernicterus in general are choreoathetoid cerebral palsy, dysfunctional upward gaze, besides sensorineural hearing loss, while comparatively cognition is spared. Continuation in addition to robust UCB hyperbilirubinemia is implicated in kernicterus in view of the capacity of the Bf to cross blood brain barrier (BBB) along with precipitate in brain in view of it being neurotoxic **[**23**]**. UCB hyperbilirubinemia can take place secondary to escalated UCB generation or lesser efficacious hepatic uptake or bilirubin conjugation. The commonest disorders of UCB hyperbilirubinemia are Criggler -Najjar syndromes (CNS)Type1 as

well as 2. The presentation of CNS Type1 individuals is in general in the form of kernicterus with total elimination of UGT1A1 activity, whereas in CNS Type2 subjects the enzyme functions partly in view of a missense mutation [24]. The toxic actions of bilirubin result in hampering DNA generation, RNA formation, protein production in the brain besides liver production in addition to changes in carbohydrate metabolism in the brain [11, 16, 23]. A model regarding inherited deficiency of bilirubin glucuronidation is represented by Gunn rat. It was a natural mutant that got invented by Dr Gunn in 1934.These rats have jaundice as well as manifest UCB hyperbilirubinemia in view of a natural mutation causing the absence of the enzyme uridine diphosphate glucuronoxylan transferase. Its transmission was in the form of a autosomal recessive property. This Gunn rat represents a natural model for bilirubin encephalopathy [25], with maximum information have been derived regarding bilirubin toxicity as well as acquisition of treatment has been obtained from studies conducted in these rats[16, 26]. Nevertheless, the Gunn rat reflects a well appreciated animal model for evaluation of besides neuronal injuring actions of hyperbilirubinemia in neonates [16, 26], however for conferring systemic protective actions of hyperbilirubinemia in adults as well[16, 27-9].

Despite, its escalated accrual can result in permanent brain injury, studies conducted recently appreciated how this bile pigment possessed numerous advantageous actions with mild escalated quantities [5, 30]. These actions have been illustrated in patients with Gilbert's Syndrome (GS). GS reflects a benign disorder that results from partial deficiency of hepatic UDP glucuronosyl transferase 1 A1(UGT1A1) without any evidence of liver injury [16, 31]. On addition of an additional dinucleotide sequence TA to the following TATA box promoter of UGT1A1(labelled as the UGT1A1* 28allele) would apparently be the commonest molecular aberration in GS. Individuals that are homozygous, illustrated equivalent to 10-35% UGT1A1 activity in contrast to normal individuals. Their presentation clinically is in the form of random hyperbilirubinemia, generally correlated with continuous fasting, fever in addition to significant physical exercise. GS represents the commonest hereditary jaundice, with its prevalence in the population being equivalent to 8%. In view of mild hyperbilirubinemia resulting in reduction in prevalence of Metabolic Syndrome (MetS (inclusive of obesity, overweight, CVD, T2D, some cancers UGT1A1 mutations might be of genetic benefits[32]. In view of that it was posited by researchers regarding the greater worldwide incidence of homozygous genetic mutations of the UGT1A1 gene might prove to be of evolutionary benefit [33]. Manipulating quantities of bilirubin might prove to be an interesting approach regarding the treatment/avoidance of metabolic diseases. Despite, Gilbert's Syndrome (GS) reflects a benign disorder, the metabolism of certain drugs which get glucuronidated might be impacted in these patients, since UGT1A1 reflects a hepatic enzyme of like non-steroidal inflammatory drugs, statins, besides human immunodeficiency

virus protease hampering agents .The decreased capacity of the hepatocytes can cause robust toxicity for the organism[33].

More recently, the Gunn rats(jj) utilization was further done for evaluation of this protection conferred by mild hyperbilirubinemia in adults [16, 27-9]. Sustenance of bilirubin quantities equivalent to the upper limits of Gilbert's individuals is illustrated, besides demonstrating escalated bilirubin quantities in tissue as well as organs throughout their lives .UCB quantities in hyperbilirubinemia homozygous Gunn rats(jj) serum (about 2.42-7.36mg/dL)[34] overlaps with the escalated bilirubin quantities observed in Gilbert's individuals[34, 35].

Additionally, recent evaluation illustrated that lesser plasma bilirubin quantities (alias 'hypo bilirubinemia' reflect probably a new pathological entity equivalent to the end of the spectrum of the ultimate hyperbilirubinemia. Plasma bilirubin quantities under 0.6 mg/dL are generally observed in patients with metabolic impairment, that might result in cardiovascular complications in addition to probably stroke [36].

Protection conferred by Bilirubin

Bilirubin has been believed to be a robust endogenous antioxidant substance. Hence more recently, the part of bilirubin in the probable avoidance of Oxidative stress(OS) – modulated diseases specifically CVD was exhaustively reviewed [2]. Provision of proof of an invivo antioxidant ability of mild hyperbilirubinemia came from the Gilbert's patients as well as Gunn rats models as detailed earlier. As per the incidence of CVD, Gilbert's patients were illustrated to possess considerably lesser prevalence (2% vs 12%) in contrast to the normobilirubinemic population [37]. The actions on lipid profiles along with cholesterol aiding in protection conferring actions of UCB have been illustrated in subjects with Gilbert's polymorphisms along with animal models of moderate hyperbilirubinemia[38]. Greater serum bilirubin has a positive correlation with an escalated high-density lipoprotein (HDL)/LDL (low density lipoprotein) ratio besides protecting these lipids from serum oxidation along with reduction in LDL cholesterol, ApoB/ApoA1, as well as very low density lipoprotein (VLDL). Furthermore, UCB might confer protection against MetS along with T2D [34, 39], along with might anticipate the propagation of DKD in T2D patients. More recently, numerous retrospective observational longitudinal studies conducted in Chinese [40, 41], Korean[42], along with Turkish [43] T2D patients illustrated that full bilirubin quantities are inversely correlated with the incidence along with propagation of DKD as well as pointed that the serum full bilirubin quantities utilization might be done in the form of a marker of DKD propagation. T2D patients possessing the normal full bilirubin

quantities in all of these studies got sub grouped into 3-4 groups as per the quartiles of the serum full bilirubin quantities at baseline. The groups possessing greater bilirubin quantities(mg/dL) in all of these studies(G3:0.6-0.9[43], Q3≥0.82[41], Q4 1.0-1.13[40], Q4≥0.88[42]), possessed the least risk of CKD propagation. These observations were validated in Chinese patients with T1D[44] . The antioxidant capacity of UCB is correlated with the redox system transforming UCB to biliverdin where there is consumption of ROS along with regeneration of bilirubin through BLVR [45]. On the other hand robust hyperbilirubinemia result in ROS generation [46], protein oxidation along with lipid peroxidation [47], resulting in apoptosis[48]in different cellular systems. Both the antioxidant along with prooxidant actions of bilirubin are validated in in vitro comparative study of Bianco etal.[49], which provided the definition of bilirubin thresholds which decided the shift amongst bilirubin actions [49].

Furthermore, anti-inflammatory along with immunomodulatory actions of bilirubin were illustrated as well [50]. These actions are a part of a stepwise processes inclusive of mild hyperbilirubinemia, ER stress, along with inflammation [1, 51]. Mild bilirubin escalation ameliorates ER stress in addition to reduction of proinflammatory cytokines [46, 52], in vivo, as well as in vitro [53]. Bilirubin illustrates a considerable anti-inflammatory ability in view of modes like hampering of adhesion molecule expression, repression of infiltration with inflammatory cells along with decreased proinflammatory cytokines quantities in animal models of endotoxemia, septicemia, along with damage from ischaemia reperfusion. Moreover, bilirubin has been illustrated to counter lipopolysaccharides (LPS), a bacterial product. LPS result in liver injury as well as CVD [52]. Additionally, UCB along with hemeoxygenase-1(HMOX1) simultaneously cause reduction of Tumor necrosis factor alpha (TNFα), nitric oxide (NO), inducible nitric oxide synthase(iNOS), endothelial impairment besides blockade of proliferation along with migration of the cells through Raf/ERK/MAPK pathway, besides illustrating anti-inflammatory actions also. Bilirubin impacts the cell signal transduction for avoidance of nuclear translocation of nuclear factor κB(NFκB) whose induction occurs by TNFα [30, 54]. ER stress in addition to the following inflammatory reaction reduction occurred subsequent to co treatment with UCB in an in vitro model of inflammation of gut [55].

More recently, Hinds etal.[56], illustrated that bilirubin possesses hormonal functions[56]. Direct binding of bilirubin to receptors implicated in energy homeostasis getting implemented (Peroxisome Proliferator Activated Receptor [PPAR], aryl hydrocarbon receptor[AhR]or constitutive and androstane receptor[CAR], events of bio transformation (like CAR, pregnane X receptor[PXR], or sensitive discernment(through MRGPRX4(Mas related G- protein coupled receptorX4))[6, 57]. Additionally, bilirubin might form complex with certain molecules implicated in energy homeostats

[58] that belongs to a lipocalin superfamily of proteins like a fatty acid binding protein [FABP1]or apolipoprotein D(apoD), along with result in activation of different extra cellular signaling pathways[6, 57].

Diabetic Kidney Disease (DKD)

DKD comprises a part of Chronic Kidney Disease (CKD) arising secondary to DM or Diabetic Nephropathy (DN)[59] takes place in general in the form of a chronic complication of DM being the commonest etiology of ESRD.As many as 40% of patients presenting with T2D propagated to DKD with the incidence of this metabolic condition escalated at a fast pace globally[60]. More recently, secondary to escalation of childhood obesity, greater prevalence of T2D is encountered in the younger population, where the manifestation is much more aggravated in contrast to adult population [62]. Additionally, escalated proof has demonstrated regarding the implications of sex along with gender variations in the variable prevalence along with particular phenotypes of DKD as well as in the influence along with regulation of general DKD risk factors. Validation of sex and gender variations in DKD while taking into account the hormonal, genetic as well as the clinical factors ensure the probability of individualized unique therapeutic strategy [62].

Clinically overt proteinuria reflects the major index of DN, however it is not the precise strategy for assessment of robustness or prognosis as numerous patients generate DKD along with renal conditions without a previous proteinuria. A biopsy of Kidney in spite of being invasive, it is necessary for a differential diagnosis of DKD along with staging the Disease [63]. Furthermore, DKD possesses the properties of hypertension, renal failure resulting in oedema along with uremic symptoms. The functional unit regarding Kidney is nephron comprised of glomerulus, proximal tubule, loop of Henle in addition to distal convoluted tubule. A proper working circulatory system allows blood to reach the glomeruli, where plasma filtration takes place in the Bowman's capsule. The human Kidney possesses the capacity of filtering 180L of blood via its glomeruli, generating about 2.1 Lurine /day [64]. DKD influences elements of both glomerulus along with tubules of nephrons, thus resulting in glomerulosclerosis, tubulointerstitial fibrosis (TIF), tubular atrophy, podocytes getting detached, along with apoptosis resulting in elimination of kidney architecture, besides renal filtration ability[65]. Initial alterations are stimulated by metabolic factors, besides being correlated with non-regulated or chronic hyperglycemia. Nevertheless, the part of variable modes have been determined inclusive of those of greater glucose quantities along with exposure to advanced glycation end-products (AGE), activation of polyol pathway, glomerular hyperfiltration, escalation of ROS, activation of diacylglycerol(DAG)/

protein kinase C(PKC) pathway, transforming growth) factorβ 1(TGF-β1) signaling , along with Renin-Angiotensin –aldosterone System(RAAS) signaling[66].

Oxidative stress(OS) along with renal ROS result in the generation of DN. Physiologically the Kidney develops a considerably significant quantities of ROS in lieu of greater metabolic action which gets balanced by a broad antioxidant system. Development of ROS takes place by variable molecules inclusive of nicotinamide adenine nucleotide (NAD(P)H) Oxidase(NOX), AGE, abnormalities in polyol pathway, uncoupling of NOS besides mitochondrial respiratory chain through Oxidative phosphorylation. At the time of pathological states like hyperglycemia, the Oxidative balance swings to a prooxidant state which aggravates tissue as well as vascular injury. Enhancement of ROS manipulates the activation of PKC, mitogen activated protein kinase (MAPK), as well as variable cytokines along with transcription factors resulting in propagation to fibrosis along with ESRD.

In the evolution of kidney disease inflammation possesses a crucial part, at the time of inflammatory event secondary to hyperglycemia, monocyte as well as lymphocytes, invade the Kidney by liberating proinflammatory cytokines along with ROS, Amplification of inflammatory reaction takes place by leukocyte activity, which facilitates cell injury, thus result in fibrosis [67]. Nuclear factor κB(NFκB), interleukin-6(IL-6), TNF-α, TGFβ soluble C-X-C chemokine ligand(CXCLs) represent cytokines as well as chemokines that play a necessary along with key part in the inflammatory reaction[68]. Clinical as well as basic studies illustrated the part of IL-6 signaling in DKD propagation. Serum IL-6 quantities are escalated in Diabetic patients with DKD vis a vis those without DKD [69], along with experimental proof pointed that IL-6 causes injury to the podocytes as DKD evolution takes place, inclusive of their hypertrophy which can cause cell cycle arrest [70].

Renal fibrosis represents end stage disease alteration in DKD. of the promoters of renal fibrosis, Hypoxia inducible factor 1 (HIF 1α) got Identified recently[71]. In case of experimental models of CKD activation of HIF 1α has been illustrated to trigger accrual of collagen along with enrolment of inflammatory cells [72]. The major stimulating factor HIF 1α activation is hypoxia, however Angiotensin II(AngII) further activates it [73]. The pro fibrotic HIF 1α pathway that result in tubulointerstitial fibrosis maturation implicates LIOX 2[74]. HIF 1α regulates biological events significant for tissue healing, wound repair along with fibrogenesis. Fibrogenesis comprises of extracellular matrix (ECM) generation along with turnover, cell adhesion, migration as well as epithelial –mesenchymal transition (EMT)[73].Of the genes controlled by HIF 1α signaling are Phosphoglycerate kinase(PGK), glucose transporter (GLUT1), vascular endothelial growth factors (VEGF), erythropoietin (EPO) Tissue inhibitors of Matrix Metalloproteinases (TIMPs)[71].

Cell demise has been illustrated to participate in escalating renal cell elimination in DKD. In vitro studies have demonstrated that hyperglycemia along with greater quantities of glucose demise result in apoptosis. Hyperglycemia that takes place in individuals with T2D stimulates aggravated apoptosis resulting in cells shrinking, chromatin condensation along with DNA fragmentation in variable cell kinds inclusive of renal proximal tubular epithelial cells.

Certain studies have observed greater glucose generation by Kidney cells possessing the capacity of upregulating proapoptotic molecules mol like Bax along with reduction of antiapoptotic molecules (B cell lymphoma-2(Bcl2), along with Bcl-xL). Control of apoptotic associated genes by greater glucose quantities is considerably complicated. In proximal tubular cells it was illustrated that greater glucose quantities along with hyperglycemia can result in induction of apoptosis in cells via control of the Bcl2/ caspase3 / poly ADP ribose polymerase (PARP) cleavage **[**75**]**. On activation caspase3 is basically implicated in the induction of PARP cleavage at the time of cell demise **[**76**]**.

Other actors illustrated to aid in cell demise in DKD are namely, Hypoxia, OS, proinflammatory cytokines, RAAS, AGE along with glucose breakdown product **[**77**]**.

Protection conferred by Bilirubin against DKD

Bilirubin posseses an avoidance influence against variable metabolic events which have been appreciated to modulate the initiation along with propagation of numerous diseases. Specifically in case of vascular diseases, DM, MetS, along with obesity. Individuals with Gilbert's presenting with mild hyperbilirubinemia possess significantly lesser risk of getting impacted by escalated OS, inflammation, or cell proliferation **[**8**]**. In addition to that homozygous Gunn rats utilize a combination of hyperbilirubinemia with considerable anti-inflammatory, anti-proliferative **[**27**]**, antihypertensive **[**28**]**, modulation of blood lipid reduction characteristic **[**78**]** along with lesser evidence of cellular senescence **[**11**]**. Despite maximum documented studies talked about experimental animal models (though not reflecting human disease) negative association of serum bilirubin quantities with DKD propagation were documented by numerous publications **[**40-3**]**. In cases of chronic hyperglycemic situations activation of variable pathways along with tissue injury take place at the time of OS, inflammation, fibrosis along with certain growth factors as well as their receptors**[**79**]**.

Nevertheless, bilirubin possesses the capacity of initiating a positive reaction over these activated along with modified pathways in DKD (fig2).

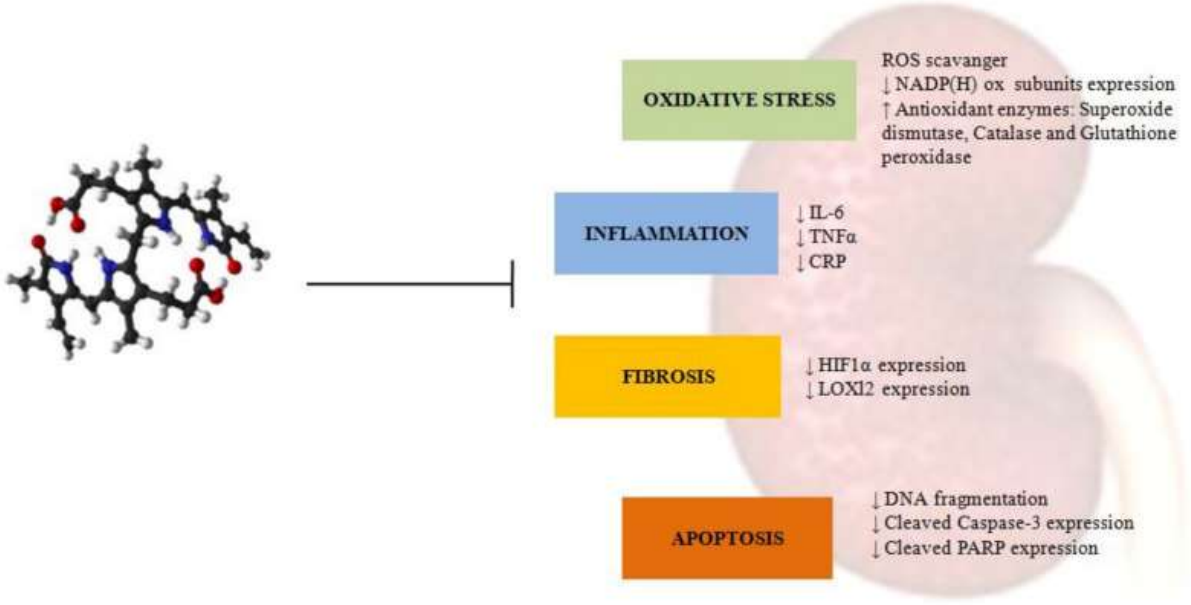

Figure 2 Courtesy ref no-9-Bilirubin protective effect on mechanisms leading to in diabetic kidney disease. ROS: reactive oxygen species; NADPH: nicotinamide adenine dinucleotide phosphate reduced form; IL-6: interleukin-6; TNFα: tumor necrosis factor alpha; CRP: C-reactive protein; HIF1α: hypoxia inducible factor 1, alpha subunit; LOXl2: lysyl oxidase like 2; DNA: deoxyribonucleic acid; PARP: nuclear poly ADP ribose polymerase.

A. Oxidative Stress (OS)

Oxidative stress (OS) in DKD reflects a key factor which combines hyperglycemia with vascular complications through two main pathways i) Metabolic modifications of targeting molecules existing in the tissue, whereas the second is the changes in the renal hemodynamics. Escalated generation of superoxide result in DNA injury, besides activation of nuclear poly ADP ribose polymerase (PARP), that causes blockade of glyceraldehyde 3 phosphate dehydrogenase (GADPH) activity, transforming early intermediates of glycolysis into pathogenic mediators **[**80**]**. Bilirubin is a robust antioxidant avoiding the actions of free radical generation as well as Oxidative injury **[**24, 34**]**. It was illustrated by Kumar etal.**[**81**]**, that serum bilirubin quantities possessed a negative association with malondialdehyde (MDA), a marker for Oxidative stress, whereas a positive correlation with superoxide dismutase(SOD), catalase(CAT), along with glutathione peroxidase (GPx), all of which reflect antioxidant enzymes**[**81, 82**]**. Vitek etal **[**83**]**, further demonstrated considerably escalated total antioxidant status in GS in contrast to healthy controls along with patients with ischemic heart disease as well as controls.

Additionally, in animal model's antioxidant are efficacious in of treatment of DKD [84]. Endogenously enhanced bilirubin of homozygous Gunn rats confer protection to the vascular compartment from the systemic OS [85]. Bilirubin results in reduction in Kidney injury in cyclosporine induction of Nephropathy [86], along with ischemia reperfusion damage [87], As far as the Gunn rat diabetic model is concerned bilirubin results in reduction of streptozocin(STZ) induced pancreatic damage injury[88]. Hampering of OS as well as downregulation of NADPH oxidase are the modes illustrated regarding protection of rodents against DKD [89]. The vasoconstrictor action of Ang II was reverted by moderate unconjugated hyperbilirubinemia, the reduction of the glomerular filtration rate (GFR) along with the escalated renal blood flow besides systolic blood pressure (BP) by foraging ROS generated by Ang II [90]. More recently, UCB impacted the intracellular Ca2+ imbalance as well as reverted the expression of vasoconstrictive pre pro endothelin that gets induced by Ang II [91]. Mild hyperbilirubinemia was further demonstrated to reduce Ang II based hypertension in mice hampering superoxide formation [92].

B. Inflammation

Weak chronic inflammation reflects one probable factor In the generation of DKD[93].Experimental observations have illustrated till date the anti-inflammatory characteristics of bilirubin in T2D. bilirubin can impact the expression quantities of cell adhesion molecules along with complement activity besides repressing the differentiation of T cells[94].The liberation of IL-2, IL-6 IL-10 as well as TNF-α is further repressed along with reduction in the expression of MHC class II in the macrophages[95]. Besides possessing the capacity of modulating the immune system, bilirubin can further impact the BLVRA enzyme [1]. Hyperbilirubinemia Gunn rats illustrate a reduction in the inflammatory reaction in LPS modulated systemic inflammation [96].

Furthermore, an inverse correlation of bilirubin C Reactive Protein (CRP), was further documented in CRP with different disease in the form of obesity [61, 85, 97].In case of Korean males, a longitudinal study evaluated the correlation amongst serum bilirubin quantities along with the prevalence of DM along with CKD in patients with DM in a Korean population. They evaluated age, sex weight, height, waist circumference (WC), history of hypertension, DM as well as smoking along with alcohol intake as the clinical parameters. Their outcomes obtained revealed an inverse correlation of serum bilirubin with extent of insulin resistance (IR), along with inflammatory markers, serum insulin, along with CRP.

Nevertheless, the anti-inflammatory along with immunomodulation actions of bilirubin are further implicated in the anti-atherosclerotic actions observed in patients with Gilbert's Syndrome[98].Keum etal.[4], recently illustrated that bilirubin nanoparticles (BRNPs), constituted of bilirubin elicits a considerably significant anti-inflammatory actions against different OS correlated diseases inclusive of ischaemia reperfusion damage, inflammatory bowel disease(IBD), experimental autoimmune encephalomyelitis, allergic lung Inflammation, psoariasis, islet xenotransplantation, that counters Inflammation by foraging escalated ROS.

C. Fibrosis

Fibrosis of the Kidney represents end stage pathological alterations in DKD. In case of animal models it was illustrated that it can be attenuated by bilirubin therapy. Escalated bilirubin quantities are correlated with superior prognosis along with lesser fibrosis generation. Moreover, bilirubin that results in reduction in the expression of fibronectin in the tubular epithelial cells in a dose-based fashion [99].

HIF 1α possesses the capacity of facilitating renal fibrosis in certain kidney diseases. Peritubular capillary elimination secondary to glomerular damage results in reduction of oxygen provision causing chronic interstitial along with tubular cells hypoxia in CKD's. Expression of HIF 1α takes place over the full Kidney, besides possessing a central part in this hypoxic reaction of tubular epithelial cells [72]. Continuous activation of HIF 1α signaling in renal epithelial cells enhances maladaptive reaction leading to induction of fibrosis as well as tissue breakdown. HIF 1α works downstream of a profibrotic signaling cascade whose initiation takes place by profibrotic Angiotensin II(AngII)in renal interstitial fibroblasts was recently correlated with a subsequent activation of the EMT along with enhanced accrual of collagen. AngII causes induction of bilirubin HIF 1α along with LOX2protein expression working in the form of a profibrotic marker [100].

Apparently, bilirubin modulates the expression of profibrotic markers. It decreases the profibrotic marker induction by AGE's as well as AngII on proximal tubular epithelial cells, along with this reduction associates with downregulation of HIF 1α transcriptional targets, LOX2 along with α smooth muscle actin(αSMA)[46]. Moreover, at the time of physiological oxygen quantities, bilirubin enhances HIF 1α mRNA transcription by reduction of ROS, besides subunits of NADPH oxidase in proximal tubular epithelial cells. Furthermore, bilirubin influences the post transcriptional manipulation of HIF 1α protein ameliorating ROS actions on the repression of the P70S6K pathway [97].

D. Apoptosis

Podocyte apoptosis might be implicated in a key part in early along with late stages of DKD, thus aiding in more reduction of the total number of podocytes along with capacity of glomerular filtration[64].PARP cleavage by caspase3 is pointed to be a cornerstone for apoptosis[76].

Hyperbilirubinemia results in reduction of AngII induction of podocyte injury by illustration of lesser DNA fragmentation, caspase3 that is cleaved along with cleaved PARP induction in Hyperbilirubinemic Gunn rats [46]. Additionally, anti-apoptotic bilirubin action was revealed in the rat model by cyclosporine induction of Nephropathy (CsA), where apoptotic cells in rat Kidney in receipt of treatment with bilirubin were considerably lesser in contrast to controls[101].Oh et al.[85], documented that CsA induced rats that were treated with bilirubin showed a block of apoptosis through upregulation of anti-apoptotic protein Bcl2 as well as downregulation of pro apoptotic Bax expression.

More recently, studies have emphasized regarding the part of renoprotective action of autophagy in case of podocytes in the DKD, Li etal.[102], illustrated that puerarin, an active substance of radix puerariae, ameliorated DKD by facilitating autophagy in podocytes. Another substance resveratrol, an antioxidant akin to bilirubin ameliorated apoptosis activating autophagy in db/db mice along with podocytes [103]. Additionally, bilirubin was detailed to possess the capacity of autophagy induction in the form of a pro- survival cell mode [104]. Furthermore, it conferred protection to podocytes in DKD models [46]. Moreover, hemeoxygenase-1(HMOX1), that reflects an antioxidant enzyme whose induction takes place in reaction to Oxidative stress (OS), facilitates autophagy besides hampering apoptosis via activation of, 5' AMP-activated protein kinase(AMPK)[105].

Conclusions Along with Future Directions

Here our concentration has been on the advantageous part of mild hyperbilirubinemia conferring protection against DKD. In the recent decade basic along with clinical studies have illustrated its actions on initiation along with propagation of renal diseases that suggest provision of actual clinical advantages with the slightest escalation of serum bilirubin quantities. Apparently endogenous bilirubin was key both in the form of a probable marker regarding propagation as well as therapeutic target for the avoidance of DKD. Patients possessing low normal full bilirubin quantities could get managed in an aggravated fashion with the idea of postponement of the propagation, besides in the form of a therapeutic target for the avoidance of CKD. researchers taking into account the probability of modulating plasma bilirubin quantities regarding avoidance of various Oxidative stress(OS) along with inflammation correlated diseases, like DKD.

References

1. Hinds TD, Stec DE. Bilirubin safeguards against cardiorenal and Metabolic diseases: a protective role in health. Curr Hypertens Rep 2019; 21:87.

2. Vitek L, Hubacek JA, Pajak A, Dorynska A, Kozela M, EremiasovaI, etal. Association between plasma Bilirubin and mortality. Ann Hepatol 2019;18:379-85.

3. Alicic RZ, Rooney MT, Tuttle KR. Diabetic Kidney Disease. Clin J Am Soc Nephrol 2017;12:2032-2045.

4. Kerum H, Kim D, Kim J, Kim TW, Whang CH, Jung W, Jon S.A Bilirubin derived nano medicine attenuates the pathological cascade of pulmonary fibrosis. Biomaterials 2021; 275:120986.

5. Vitek L. The role of Bilirubin in diabetes, Metabolic Syndrome and cardiovascular disease. . Front Curr Pharmacol 2012; 3:55.

6. Vitek L. Bilirubin as signaling molecule. Med Res Rev 2020; 40:1335-51.

7. Fevery J. Bilirubin in Clinical practice.: a review. Liver Int Off J Int Assoc Study Liver 2008; 28:592-605.

8. Vitek L, Bellarosa C, Tiribelli C. Induction of mild hyperbilirubinemia: hype or real therapeutic opportunity.? Clin Pharmacol Ther 2019;106:568-75.

9. Bianco A, Tiribelli C BellarosaC. Translational approach to the protective effect of bilirubin on Diabetic Kidney Disease. Biomedicines 2022; 10:696.

10. Hahm JS, Ostrow JD, Mukherjee P, Celic I. Ionization and self-association of unconjugated bilirubin, determined by rapid solvent partition from chloroform with further studies of bilirubin solubility. J Lipid Res 1992:33:1123-37.

11. Zelenka J, Dvdrak A, Alan L, Zadinova M, Haluzik M, Vitek L. Hyperbilirubinemia protects against aging associated inflammation and Metabolic deterioration. Oxid Med Cell Longev 2016; 2016: 6190609.

12. Sundar Raghavan VL, Sindhwani P, Hinds TD. Glucuronidation and UGT isoenzymes in bladder: new targets for the treatment of urothelial carcinoma. Oncotargets 2016; 8:3640-8.

13. Mandez Sanchez N, QIZ, Vitek L, Arrese M. Evaluating an outdoor patient with an elevated Bilirubin. Am J Gastroenterol2019; 114:1185-8.

14. Tiribelli C, Ostrow JD. Intestinal flora andbilirubin. J Hepatol 2005;42:170-2.

15. Sticova E, Jirsa M. New insights in bilirubin metabolism andtheir Clinical implications. World J Gastroenterol 2013;19:6398-407.

16. Vitek L, Ostrow JD. Bilirubin chemistry and metabolism: harmful and protective effects. Curr Pharm Des 2009;15: 2869-83.

17. Kulvinder Kochar Kaur, Allahbadia GN, Singh M. Potential role of Epigenetic Modulation in prevention or therapy for Diabetic Kidney Disease-still a dream or a reality –A Systematic Review' 'J Diab Nephro Diab Mgmt 2021:1:1(1-26).

18. Kulvinder Kochar Kaur, Allahbadia GN, Singh M. An update in the utilization of N-acetylcysteine & vitamin c for tackling the oxidative stress in acute kidney injury secondary to robust sepsis - A systematic review. J Clini Nephrol.2022; 6: 001-018DOI: 10.29328/journal.jcn.1001084.

19. Kulvinder Kochar Kaur, Allahbadia GN, Singh M. Potential role of epigenetic modulation in prevention or therapy for Diabetic kidney disease-still a dream or a reality; keynote presentation;2021; at the 3rd Annual Summit on Diabetes, Obesity and Heart held during December 10, 2021, in Webinar by Allied Academies and the Editors of Journal of Cardiovascular Medicine and Therapeutics, Journal of Cholesterol and Heart Disease & Journal of Diabetology.

20. Kulvinder Kochar Kaur, Allahbadia GN, Singh M. Can Vitamin D Supplementation prevent / delay/ halt the progression of Diabetic nephropathy; A Systematic Review on mechanism of Vitamin D Crosstalk with Vitamin D Receptor, with others like Megalin- Cubilin and Amnio less Complex along with FGF23-Klotho Complex.'' J Clin Diabetes Obes 2021.

21. Kulvinder Kochar Kaur, Allahbadia GN, Singh M. 'An Update on the Approaches of Avoidance of Propagation of Chronic Kidney Disease resulting in Reversal or Prevention of Kidney transplantation-A Systematic Review''. J Clini Nephrol. 2022; 6: 040-057.DOI: 10.29328/journal.jcn.1001089.

22. Kulvinder Kochar Kaur, Allahbadia GN, Singh M. 'An update on etiology of Chronic Kidney Disease with role of Associated Vitamin K deficiency in Prevention of Vascular calcification& cardiovascular risks & avoid mortality: A minireview' 'Acta Scientific Nutritional Health2022; 6 (5):1-10.

23. Reddy DK, Pandey S. Kernicterus. In Stat Pearls, Stat Pearls Publishing: Treasure Islands, FL, USA, 2021.

24. Booms AC, Bulmer AC, Coombes JS, Fassett RG. Circulating bilirubin and defense against kidney disease and cardiovascular mortality mechanisms contributing to protection in Clinical investigations. Am J Physiol Renal Physiol 2014;307: F123- F136.

25. Schutta HS, JohnsonI.Bilirubin encephalopathy in the Gunn rat:a fine structure study of the cerebellar Cortex .JNeuropathol Exp Neurol 1967;26:377-96.

26. Gazzin S, Zelenka J, ZdrahalovaL, Konickova R, Zabetta CC, Giraudi PJ, etal. Bilirubin Accumulation and Cyp mRNA expression in selected brain regionsof jaundiced Gunn rat pups. Pediatr Res 2012; 71:653-60.

27. Ollinger R, Bilban M, EratA, Froio M, McDaid J, Tyagi S, etal. Bilirubin: a natural inhibitor of vascularsmooth muscle cells proliferation. Circulation 2005; 112:1030-9.

28. Pflueger A, Croatt AJ, Peterson TE, Smith LA, De'Uscio LV, Karusic ZS, Nath KA. The hyperbilirubinemic Gunn rat is resistant to the pressor effects of AngiotensinII. Am J Physiol Renal Physiol 2005;288:F552- F558.

29. Zelenka J, Muchova L, Zelenkova M, Vanova K, Vreman FIJ, Wong RJ, Vitek L. intracellular Accumulation of Bilirubin as a defense mechanism against Oxidative stress. Biochimie 2012; 94:1821-7.

30. Kang SJ, Lee SC, Kruzliak P. Effects of serum bilirubin atherosclerotic processes. Ann Med 2014;46:138-47.

31. Wagner KH, Wallner M, Molzer C, Gazzin S, Bulmer AC, Tiribelli C, Vitek L. Looking to the horizon: the role of Bilirubin in the development and prevention of age related chronic disease. Clin Sci Lond Eng 1979 2015; 129:1-25.

32. Wagner KH, Shiels RG, Lang CA, Seyed Khoei NA, Bulmer AC. Diagnostic criteria and contributors to Gilbert's Syndrome. Crit Rev Clin Lab Sci 2018;55:129-39.

33. Erlinger S, Arias IM, Dhumeaux D. Inherited disorders of bilirubin transport and conjugation: new insightinto molecular mechanism and consequences. Gastroenterology 2014; 146:1625-38.

34. Boon AC, Hawkins CL Bisht K, Bakrania B, Coombes JS, Wagner KH, Bulmer AC. Reduced Circulating oxidized LDL is associated with hypocholesterolemia and enhanced thiol status in Gilbert's Syndrome. Free Radical Biol Med 2012; 52: 2120-7.

35. Bulmer AC, Blanchfield JT, Toth I, Fassett RG, Coombes JS. Improved resistance to serum oxidation in Gilbert's Syndrome :a mechanism for cardiovascular protection. Atherosclerosis 2008; 199:390-6.

36. Creeden JF, Gordon DM, StecDE, Hinds TD. Bilirubin as a metabolic hormone:the physiological relevance of low levels. Am J Physiol Endocrinol Metab 2021;320:E191-E207.

37. Lee SJ, Jee YH, Jung KH, Hong S, Shin ES, Jee SH. Bilirubin and stroke risk using a Mendelianrandomization design. Stroke 2017; 48:1154-60.

38. Hinds TD, Stec DE. Bilirubin, a cardiometabolic signaling molecule. Hypertens Dallas Tex 1979 2018; 72:788-95.

39. Stec DE, JohnK, Trabbic CJ, Luniwal A, Hankins MW, Baum J, Hinds TD. Bilirubin binding to PPARγ inhibits lipid accumulation. PLoSONE 2016;11: e0153427.

40. Chan WK, Tsai SS, LiYR, Chou WY, Chen HL, Chen ST. Association between serum bilirubin levels and progression of albuminuria in Taiwanese with type2 Diabetes mellitus. Biomed J 2021; 44:201-8.

41. Liu M, Li J, Lv X, HeY. Bilirubin and its changes were negatively associated with Diabetic Kidney Disease incidence and progression: a 5 year cohort study on 5323 Chinese male Diabetic patients. J Diabetes Complic 2018;32:1012-17.

42. Ahn KH, Kim SS Kim WJ, Kim JH, Nam YJ, Park SB, etal. Low serum bilirubin levels predicts the development of Chronic Kidney Disease in patients with type2 diabetes mellitus. Korean J Intern Med 2017;32:875-82.

43. Uludag K, Oguzhan N, Arikan T, Boz G. Serum bilirubin level and its impact on the progression of Chronic Kidney Disease. Int Urol Nephrol 2018;50:1695-1701.

44. LiX, Zhang L, ChenH, GuoK, Yu H, ZhouJ, etal. Relationship between serum bilirubinConcentration and Diabetic Nephropathy in Shanghai Han's patients with type1 diabetes mellitus.BMC Nephrol 2017; 18:114.

45. Rigato I, OstrowJD , Tiribelli C. Bilirubin and the risk of common non hepatic diseases. Trends Mol Med 2005;11: 277-83.

46. Bianco A, Pinci S, Tiribelli C, BellarosaC. Life long hyperbilirubinemia exposure and bilirubin priming in vitro Metabolic damage. Front Pharmacol 2021; 12:411.

47. Kumar S, GuhaM, Choubey V, Maity P, Srivistava K, Puri SK, etal. Bilirubin inhibits Plasmodium falciparum growth through the generation of Reactive oxygen species . Free Radical Biol Med 2008; 44: 602-13.

48. Oakes GH, Bend JR. Early steps in bilirubin mediated apoptosis in murine hepatoma(Hepa1c.1c7)cells are characteristized by aryl hydrocarbon receptor independent Oxidative stress and activation of the mitochondrial pathway. J Biochem Mol pharmacol Toxicol2005; 19: 244-55.

49. Bianco A, Dvorak A, Capkova N, Gironde C, Tiribelli C, Furger C, etal. The extent of intracellular accumulation of bilirubin determines its anti or prooxidant effects. Int J Mol Sci 2020;21:8101.

50. Jangi S, OtterbeinL, Robson S. The molecular basis for the immunomodulatory activities of unconjugated bilirubin. Int J Biochem Cell Biol 2013; 45: 2843-51.

51. Barateiro A, Vaz AR, Silva SL, Fernandes A, Brites D.ER Stress, mitochondrial dysfunction and Calpan /JNK activation are involved in oligodendrocytes precursor Cell Death by unconjugated bilirubin. Neuromolecular Med 2012; 14: 285-302.

52. Tran DT, JeongYY, Kim JM, Bae HB, Son SK, Kwak SH. The anti-inflammatory role of bilirubin on ‘’Two Hit’’ sepsis animal model. Int J Mol Sci 2020; 21:8650.

53. Li Y, Huang B, YeT, Wang Y, Xia D, Qian J. Physiological Concentration of bilirubin control inflammatory responseby NFκB activation. Intimmunopharmacol 2020; 84:106520.

54. Mazzone GL. Rigato I, Ostrow JD, Tiribelli C. Bilirubin effects on endothelial adhesion moleculeis mediated by nuclear factor κB signaling pathway.Biosci Trends 2009; 3: 151-7.

55. Gundamaraju R, Vemuri R, Chong WC, Bulmer AC, EriR. Bilirubin attenuates ER Stress mediated inflammation, escalates apoptosis and reduces proliferation in the LS174T colonic epithelial cell lines. Int J Med Sci 2019;16:135-44.

56. Hinds TD, Gordon DM, Combs SD, Stec DE. Bilirubin, a novel endocrine hormone with fat burning properties. FASEB J2018; 32:605.6.

57. Vitek L, Tiribelli C. Bilirubin :the yellow hormone? Hepatol 2021.

58. Ziberna L, Jenko PraznikarZ, Petelin A. Serum bilirubin level in obese and overweight individuals. The importance of anti-inflammatory and antioxidant responses. AntioxidBasel Switz 2021;10:1352.

59. Persson F, Rossing P. Diagnosis of Diabetic Kidney Disease:starte of the art and future perspectives. Kidney IntSuppl 2018; 8:2-72.

60. Slyne J, Slattery C, McMarrow T, R MP.New developments concerning the proximal tubulein Diabetic Nephropathy: in vitro models and mechanisms . Nephrol Dial Transplant 2015;30:iv 60- iv.

61. Amatruda M, Gembillo G, Giuffrida AE, Santoro D, Conti G.The aggressive Diabetic Kidney Disease in youth onset type2 diabetes: pathogenetic mechanisms and potential, therapies. Medicina 2021; 57:868.

62. Giandalia A, Giuffrida AE, Gembillo G, Cucinotta D, Squadritto G, Santoro D, etal. gender differences in Diabetic Kidney Disease :focus on hormonal , genetic and Clinical factors. Int J Mol Sci 2021; 22:5808.

63. Santoro D, Torreggiani M, PellicanoV, Cernaro V, Messina RM, Longhitano E, etal. Kidney biopsy in type2 Diabetic patients:critical reflections on present indications and diagnostic al is alternatives . Int J Mol Sci 2021; 22:5425.

64. Bose M, AlmasS, Prabhakar S. Wnt signaling and podocyte dysfunctionin Diabetic Nephropathy. J Investig Med Off Publ Am Fed Clin Res 2017; 65: 1093-1101.

65. Maezawa Y, Takemoto M, Yokote K.Cell biology of Diabetic Nephropathy:role of endothelial cell tubulointerstitial cells and podocytes. J Diabetes Investig 2015; 6:3-15.

66. Xiong Y, Zhou L. The signaling of cellular senescence in Diabetic Nephropathy . Oxid Med CellLongev 2019; 2019: 7495629.

67. Duran SelgadoMB, Rubio GuerreraAF. Diabetic Nephropathy and inflammation. World J Diabetes 2014;5:393-8.

68. Talsma DT, Katta K.EttemaMAB, Kel B, KuscheGullbergM, Daha MR, etal. Endothelial hyalurasulphate deficiency reduces Chronic inflammation and fibrosis in murine Diabetic Nephropathy .Lab Investig 2018;98:427-38.

69. Taslipinar A, Yaman H, Yilmaz MI, Demirbas S, Saglam M, Taslipinar MY, etal. The Relationship between inflammation, endothelial dysfunction and proteinuriain patients with Diabetic Nephropathy .Scand J ClinLab Investig 2011;71:606-12.

70. Feigerlova EV, BattagliaHsu SF.IL-6 signaling in Diabetic Nephropathy :from pathophysiologyto therapeutic perspectives. Cytokine Growth Factor Rev 2017;37:57-65.

71. Hasse VH. Hypoxia inducible factor 1 signaling in the development of Kidney fibrosis. Fibrogenesis Tissue Repair 2012;5:S16.

72. Kimura K, Iwano M, Higgins DF, Yamakuchi Y, Nakatani K, Harada K, etal.Stable expression of HIF 1α in tubular epithelial cells promotes interstitialfibrosis. Am J Physiol Renal Physiol 2008;295:F1023-F1029.

73. Schietke R, Warnecke C, WI.S I, Schodel J, MoleDR, CampeanR, etal.The Lysyl OxidaseLOX and LOX2 are necessary and sufficient torepress E cadherin in Hypoxia: insightsinto intracellular transformation processes mediated by HIF. JBiol Chem 2010;285:6658-69.

74. Huang H, Fan Y, GaoZ, Wang Z, ShaoN, ZhangL, etal. HIF 1α contributes to AngII inflammatorycytokine production in podocytes.BMC Pharmacol Toxicol 2019;20:59.

75. Habib SL. Diabetes and renal tubular epithelial cells apoptosis. World J Diabetes 2013;4:27-30.

76. Jagtap P, Ssazo C. Poly (ADP ribose) polymerase and the therapeutic effects of its inhibitors. Nat Rev Drug Discov 2005;4:421-40.

77. Cardosa VG, Goncalves GL, CostaPessoa JM, T K, Linx BB, Casare FAM, etal. Angiotensin II induced podocyte apoptosis is mediated by endoplasmic reticulum stress/PKC-δ/P38/MAPK pathway activation and trough increasedNa+/H+Exchange isoform 1 activity. BMC Nephrol 2018;19:179.

78. Wallner M, Marculescu R, Doberer D, Wolzt M, Wagner O, Vitek L, etal. Protection from age related lipid biomarkers and inflammation contributes to cardiovascular Protection in Gilbert's Syndrome. Clin Sci Lond Eng 1979 2013;125:257-64.

79. BahreiniE, RazeiChianehY, Nabi AfzadiM.Molecular mechanisms involved inintrarenal Renin-Angiotensin and alternative pathways in Diabetic Nephropathy .– A review. Rev Diab Stud RDS 2021;17:1-10.

80. Yuan T, Yang T, ChenH , FuT, Hu Y, WangJ, etal. NewInsights into Oxidative stress and inflammation during diabetes mellitus accelerated atherosclerosis. Redox Biol 2018; 20:247-60.

81. Kumar A, Pant P, Basu S, Rao GRK, Khanna HD. Oxidative stress in neonatal hyperbilirubinemia.J Trop Pediatr 2007;53:69-71.

82. YanP, Zhang Z, Miao Y, Xu Y, Zhu J, Wan Q.Physiological serum bilirubin total Concentrations were inversely associated with peripheral Diabetic Neuropathy in Chinese patients with type2 diabetes:a crossectional study. Diabetol Metab Syndr 2019;11:100.

83. Vitek L, Jirsa M, Brodanova M, Kalab M, Danzing V, etal. Gilbert's Syndrome and ischaemic heart Disease:a Protective effects of elevated bilirubin levels. Atherosclerosis 2002; 160:449-56.

84. Jha JC, Banal C, Chow BSM, Cooper ME, Jandeleit Dahm K. Diabetes and Kidney Disease: Role of Oxidative stress. Anti Oxid Redox Signal 2016; 25:657-84.

85. Boon AC, Lam AK, Gopalan V, Benzie LF, Briskey D, Coombes JS, Fassett RG, Bulmer AC. Endogenously elevated bilirubin modulates Kidney function and from circulating Oxidative stress in a rat model of adenine induced Kidney failure. Sci Rep 2015; 5:15482.

86. Oh SW, Lee ES, Kim S, Na KY, ChaeDW, Kim S, etal. Bilirubin attenuates the renal tubular injury by inhibition of Oxidative stress and apoptosis. BMC Nephrol 2013; 14:105.

87. Adin CA, Croker BP, Agarwal A.Protective effects of exogenous bilirubin on ischaemia reperfusion injury in theisolated perfused rat Kidney. Am J Physiol Renal Physiol 2005;288:F778-F784.

88. Fu YY, Kang KJ, A JM, Kim HR, Na KY, Chae DW, Kim S, etal. Hyperbilirubinemia reduces the streptococci(STZ), induced pancreatic damage attenuating Oxidative stress in the Gunn rat .Tohoku J Exp Med 2010; 222:265-73.

89. Fujii M, Inoguchi T, Sasaki S, Maeda Y, Zheng J, Kobayashi K, etal. Bilirubin and biliverdin protect rodents against Diabetic Nephropathy by downregulating NADPH Oxidase. Kidney Int 2010; 78:905-19.

90. Vera T, Stec DE. Moderate hyperbilirubinemia renal hemodynamics in Ang II dependent hypertension. Am J Physiol Regul Integr Comp Physiol 2010;299: R1044-R1049.

91. Stec DE, Hosick PA, Granger JP. Bilirubin and renal hemodynamics and blood pressure. Front Pharmacol 2012; 3:18.

92. Stec DE, Storm MV, Pruett BE, Gousset MU. Antihypertensive actions of Moderate hyperbilirubinemia: role of superoxide inhibition. Am J Hypertens2013; 26:918-23.

93. Herder C, Kannenberg JM, Huth C, Carstensen Kirberg M, Rathmann W, KoenigW, etal.Pro inflammatorycytokines predict the incidence and progression of distal sensorimotor peripheral Neuropathy.KORA F4/FF4 Study. DiabetesCare 2017;40:569-76.

94. Chen J, Wang J, Zhang X, Zhu H. Inverse association between total serum bilirubin levels and Diabetic foot in Chinese patients with type2 diabetes mellitus. Med Sci Monit 2017;23:5916-23.

95. Jin J, Wang W, Gu T, Chen C, Sun J, Chen W, etal. Low serum bilirubin levels contribute to the presence and progression of distal symmetrical peripheral Neuropathy in Chinese patients with type2 diabetes mellitus. Diabetes Metab 2019;45: -47-52.

96. Valaskova P, Dvorak A, Lenicek M, Zizalova K, Kutinova Canova N, Zelenka J, etal. Hyperbilirubinemia in Gunn rats is associated with decreased inflammatory response in LPS mediated systemic inflammation. Int J Mol Sci 2019; 20:2306.

97. Kim SG, Ahn SY, Lee ES, Kim S, Na KY, Chae DW, Chin HJ. Bilirubin activates transcription of HIF 1αin proximal tubular epithelial cells cultured in the physiological oxygen content. J Korean Med Sci 2014;29:S146.

98. Bulmer AC, Bakrania B, Du Toit EF, Boon AC, Clark PJ, Powell LW, etal. Bilirubin acts as a multipotent guardian of cardiovascular integrity: more than just a radical idea. Am J Physiol Heart Circ Physiol 2018;315:H429-H447.

99. Park S, Kim DH, Hwang JH, Kim YC, Kim JH, Lim CS, etal. Elevated bilirubinlevelsare associated with a better renal prognosis and ameliorates Kidney Fibrosis. PLoSONE 2017;12: e0172434.

100. Wang Z, Tang L, Zhu Q, YiF, ZhangF, LiPL, etal. Hypoxia inducible factor 1 contributes to the profibrotic action of Angiotensin II in renal medullary interstitial cells. Kidney Int 2011; 79:300-315.

101. Oh SW, AhnJM, LeeYM, Kim S, Chin HJ, ChaeDW, Na KY. Activation of Hypoxia inducible factor 1 by cobalt is associated with the attenuation of tissue injury and cyclosporine induced Nephropathy. Tohoku J Exp Med 2012; 226:197-206.

102. LiX, Zhu Q, Zheng R, Yan J, Wei M, Fan Y, etal. Puerarin attenuates Diabetic Nephropathy by promoting autophagy in podocytes. Front Physiol 2020; 11:73.

103. Huang SS, Ding DF, Chen S, Dong CL, Ye XL, Yuan YG, etal. Resveratrol protects podocytes against apoptosis via stimulation of autophagy in a mouse model of Diabetic Nephropathy. Sci Rep 2017; 7:45692.

104. Qaisya M, Mardesic P, Pastore B, Tiribelli C BellarosaC. The activation of autophagy protect neurons and astrocytes against bilirubin induced cytotoxicity. Neurosci Lett2017; 661:96-103.

105. Dong C, Zheng H, Huang S, You N, Xu J, Ye X, etal. Hemeoxygenase-1 enhances autophagyin podocytes as a protective mechanism against high induced apoptosis.Exp Cell Res 2015;337:146-59.