Arch Clin Exp Med 2020;5(2):79-85. e-ISSN: 2564-6567

DOI:10.25000/acem.699424 Derleme / Review

# Bilirubin metabolism and its role in atherosclerosis

## Bilirubin metabolizması ve aterosklerozdaki rolü

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Abstract

Hemoglobin is not an guiltless bystander of the pathophysiology in a number of atherosclerotic diseases. Heme, which is released from hemoglobin or other heme proteins, triggers various pathophysiological consequence, including heme stress as well as intracellular stress. Although heme serves key functions and is tightly controlled, high levels of free heme, which may occur in various pathophysiological conditions, are may hazardous via pro-oxidant, pro-inflammatory, and cytotoxic effects.

Heme oxygenases are heat shock protein enzymes that use heme as a substrate and function as an essential antioxidant adaptive response by all human cells. A major function of heme oxygenases is clearance of heme that accumulate in tissues due to erythrocyte turnover. The potentially toxic free heme is converted by heme oxygenases into carbon monoxide, iron, and biliverdin, the third of which is reduced to bilirubin. In literature the heme degradation pathway has been demonstrated to play a protective role against the development of atherosclerosis. Because growing evidence suggests that oxidative stress is involved in atherosclerosis.

This review documents the roles of bilurubin in atherosclerosis and focuses on the clinical significance as a potential therapeutic target in atherosclerotic diseases, such as coronary artery disease.

Keywords ; Bilirubin, Atherosclerosis, Heme oxygenases, Oxidative stress, Cardiovascular diseases.

Öz

Hemoglobin bir dizi aterosklerotik hastalığın patofizyolojisinde suçsuz bir seyirci değildir. Hemoglobinden veya diğer hem proteinlerinden salınan hem hücre içi stresin yanında hem stresi de dahil olmak üzere çeşitli patofizyolojik sonuçları tetikler. Hemin anahtar fonksiyonları olması ve sıkı bir şekilde kontrol edilmesine rağmen, çeşitli patofizyolojik koşullarda ortaya çıkabilen yüksek serbest heme seviyeleri, pro-oksidan, pro-enflamatuar ve sitotoksik etkiler nedeniyle tehlikeli olabilir.

Hem oksijenazlar, hemi bir substrat olarak kullanan ve tüm insan hücreleri tarafından gerekli bir antioksidan adaptif yanıt olarak işlev gören ısı şoku protein enzimleridir. Hem oksijenazların en önemli fonksiyonu eritrosit döngüsüne bağlı dokularda biriken hemin temizlenmesidir. Potansiyel toksik serbest hem hem oksijenazları tarafından karbon monoksit, demir ve biliverdine dönüştürülür, bunlardan üçüncüsü bilirubine indirgenir. Literatürde hem degradasyon yolunun ateroskleroz gelişimine karşı koruyucu bir rol oynadığı gösterilmiştir. Çünkü artan kanıtlar oksidatif stresin aterosklerozda rol oynadığını düşündürmektedir.

Bu derleme bilurubinin aterosklerozdaki rollerini belgelemekte ve koroner arter hastalığı gibi aterosklerotik hastalıklarda potansiyel bir terapötik hedef olarak klinik öneme odaklanmaktadır.

Anahtar Kelimeler; Bilirubin, Ateroskleroz, Hem oksijenaz, Oksidatif stres, Kardiyovasküler hastalıklar

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Conflict of Interest: No conflict of interest was declared by the authors.
Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.

Financial Disclosure: The authors declared that this study has received no financial support.
Finansal Destek: Yazarlar bu çalışma için finansal destek almadıklarını beyan etmişlerdir.

Geliş Tarihi / Received: 07.03.2020
Kabul Tarihi / Accepted: 14.07.2020
Yayın Tarihi / Published: 31.08.2020

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In this review we aimed to examine several experimental and clinical studies investigating the relationship between atherosclerotic diseases and bilirubin and the effects of bilirubin on the atherosclerotic process. It is known that oxidative stress is involve in atherosclerosis. Heme degradation pathway which bilirubin is synthesised has been demonstrated to play a protective role against the development of atherosclerosis. The protective properties of bilirubin are observed at every stage of the atherosclerosis process. This review documents the roles of bilurubin in atherosclerosis and focuses on the clinical significance as a potential therapeutic target in atherosclerotic diseases.

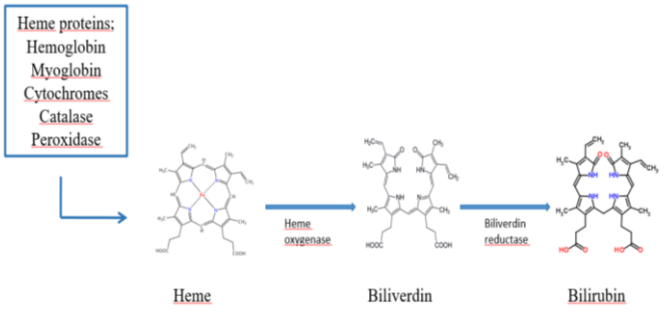
**Bilirubin**

Bilirubin become of four pyrrole rings, both connected by carbon bridges formed during its catabolism. From an evolutionary perspective, protoporphyrin ring is a exclusive metal chelator with outstanding properties. In contrast, for many years, bilirubin had been thought to be a toxic waste product, especially for the central nervous system [1]. Thus bilirubin often has been considered as a metabolic waste. However, recent evidence suggests that bilirubin may have many other beneficial effects, including anti-oxidant effects, anti-inflammatory effects, and direct effects on cell signaling [2]. Today many epidemiological data highlight that bilirubin may play a protective role against atherosclerosis [1].

**Bilirubin Synthesis**

The bulk of bilirubin is caused by the breakdown of red blood cells that fill its life. Approximately 85% of total bilirubin is originated from hemoglobin in mature red blood cells destroyed in reticuloendothelial cells. The remaining 15% consists of immature red blood cells that break down in the bone marrow and other molecules such as; myoglobin, cytochromes, catalase and peroxidase [3]. When both pathways reach the oxygenase enzyme system, iron is usually upgraded to ferric (Fe+3) form and Hemin is formed. Hemin is reduced to NADPH, switching to become iron ferro (Fe+2) form. Then porphyria with more oxygen is added to the alpha text bridge between the pyrrole rings, and ferro iron is oxidized again in the form of ferri. Finally with the addition of oxygen, iron is released, carbonmonoxide is formed, biliverdin is revealed. Biliverdin by reducing the text bridge between the pyrrole rings to the methylene group, it forms bilirubin, a yellow pigment [4]. Bilirubin formation pathways are shown in figure 1.

Figure 1. Bilirubin synthesis.




The first formed bilirubin is known as indirect bilirubin (free bilirubin, unkonjuge bilirubin). Indirect bilirubin is insoluble in water, does not pass through urine and is not excreted with bile. Unconjuge bilirubin is usually carried to the liver through circulation by binding to albumin [3]. Serum albumin-bound bilirubin comes to liver, leaving albumin in the hepatocyte sinusoidal membrane and passing through the membrane. Bilirubin entering the liver cell binds reversibl to soluble proteins known as Ligandins or Protein Y (glutathione-S-transferase gene family). Hepatocytes add glucuronic acid molecules to bilirubin, transforming it into a polar form conjuge bilirubine that can be easily excreted into bile. This process is called conjugation. The conjugation of bilirubin is catalysed by a specific glucuronosyltransferase in the endoplasmic reticulum, uses UDP-glucuronic acid as the glucronosyl transmitter, and is defined as bilirubin-UGT [3] (Figure 2).

Firstly bilirubin monoglucuronide is an intermediate and then decoded into diglucuronide. Most of bilirubin thrown into bile is in the form of bilirubin diglucuronide. However, when bilirubin conjugates are abnormally present in human plasma, they are mostly in the form of monoglucuronids. Conjugated bilirubinin secretion of bile is known as the rate-limiting step in bilirubin metabolism. This transport process takes place through the active transport mechanism via MRP-2 (multidrug resistance-like protein 2). When conjugated bilirubin reaches the terminal ileum and large intestine, glucronides with β-glucronidases are removed and urobilinogens, which are colorless tetrapyrol compounds, are formed [4].

Under physiological conditions, the dominant circulation form of bilirubin is the un-conjugated albumin-bound form but four forms of bilirubin have been isolated in the serum. Non conjugated-bilirubin (α-bilirubin) 27%, mono-conjugated bilirubin (β-bilirubin) 24%, conjugated bilirubin, Di bilirubin (γ-bilirubin) 13% protein, which is connected and 37% irreversible bilirubin (δ-bilirubin) in serum. Antioxidant activity and cardioprotective potential can be attributed to any of the bilirubin forms [5].

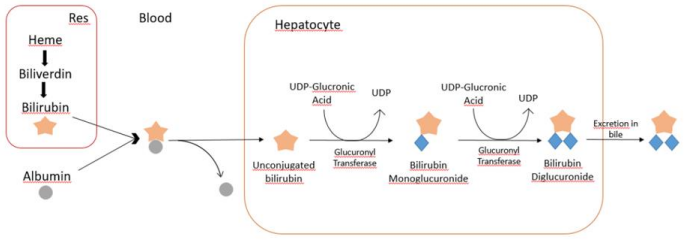
**Endogenous Antioxidant Effects Of Bilirubin**

Bilirubin was for many years considered a waste product of oxygenases. While much attention was not shown to the physiological roles of bilirubin until the 1987 year studies showing that bilirubin suppresses the oxidation of peroxyl radicals more than α-tocopherol [6]. Today, bilirubin's antioxidant potential has been confirmed by additional studies; it has been shown that bilirubin is 20 times more effective in preventing LDL oxidation than Trolox [7]. It has also been reported that non-conjugated bilirubin concentrations as low as 10 nmol/L protect neuronal cultures from oxidative stress [8]. Nowadays bilirubin is considered the most potent endogenous antioxidant due to its continuous improvement in the bilirubin / biliverdin redox cycle, resulting in both in vitro and in vivo protective lipid peroxidation. Besides this antioxidative effect, bilirubin also exerts immunosuppressive effects on antigen presenting cells and T cells, as well as on inhibition of adhesion molecule expression and immune cell migration. These concepts might form the basis of a new understanding of bilirubin metabolism, creating a new treatment approach for atherosclerosis, cancer, autoimmune diseases [9].

**Bilirubin as an anti-atherogenic molecule**

Undoubtedly atherosclerosis is a leading cause of death in developed and developing countries. Yet, well known labile free heme is one of the many known risk factors for atherosclerosis and contributes to the formation of this complex disease. Nowadays total bilirubin in circulation is known to be inversely and independently associated with the risk of future cardiovascular heart disease (CHD). A large number of clinical trials have strongly demonstrated the protective role of bilirubin against atherosclerotic heart diseases. Undoubtly higher total bilirubin contributes to lower CHD risk [10].

Figure 2. Bilirubin diglucuronide synthesis in hepatocytes.




Res: Reticulo-endothelial system, UDP: Uridine diphosphate

In 1994, Schwertner et al. they were the first researchers to report a negative relationship between lower serum bilirubin concentrations and CHD on as many as 900 men. The strength of this relationship was found to be similar to smoking, hypertension and dyslipidemia. In this pioneering study, a 50% decrease in total bilirubin was associated with a 47% increase in the likelihood of more severe coronary artery disease, which was proven by coronary angiography [11]. Later, Vitek et al. they started a retrospective study on cases with Gilbert Syndrome, known as benign hyperbilirubinemia and characterized by slightly increased unkonjuge hiperbilirubinemia. Although the cohort was small (n = 50), individuals with benign hyperbilirubinemia were found to have a lowest 2% prevalence rate for CHD compared with the general population. This study was continued prospectively and patients were followed up for 3 years. While there was a 3,1% incidence of CHD in control subjects during this period, no cases of were seen in patients with Gilbert Syndrome [12].

A year later, a meta-analysis involving 11 studies by Novotny and Vitek were reported strong negative correlation between serum bilirubin levels and atherosclerosis severity in men (p<0,0001). Also, nonparametric regression analyses have shown a negative relationship between serum bilirubin concentrations and atherosclerotic diseases. In a one study, it was found that every 1,0 µmol/L increase in serum bilirubin was associated with a 6,5% decrease in cardiovascular disease [13]. The same relationship was also demonstrated in a study on patients with cardiac X syndrome, which was followed up for 5 years. Patients with the lowest serum bilirubin levels were reported to have a higher incidence of hospitalization for non-fatal myocardial infarction, ischemic stroke, unstable angina [14]. Indeed lower serum bilirubin was able to independently estimate long-term mortality in coronary artery disease (CAD) (HR 0.34, 95% CI 0.16-0.70) and unstable angina (UAP) (HR 0.49, 95% CI 0.31-0.78) groups [15].

It has also been reported to be associated bilirubin with coronary artery calcification (CAC), which is a good indicator of the presence and amount of coronary atherosclerosis. Because in a cross-sectional study of 398 men and 239 women, serum bilirubin concentrations were found to be strongly associated with CAC scores and independent predictors of CAC in both men and women [16]. Similarly in a cross-sectional study conducted on 2682 non-CAD patients for bilirubin was found to be inversely related to total coronary plaque. In this prevalence of coronary atherosclerosis or >50% coronary artery stenosis was found to be lower in people with high serum bilirubin levels (>1,2 mg/dL) than those with normal serum bilirubin levels [17]. As a result; serum bilirubin, creates antioxidant-antilipoperoxidative effects in atherosclerotic plaques and appears to be negatively associated with the severity of atherosclerosis [18].

In addition to these studies, there are studies that suggest a relationship between serum bilirubin levels and general mortality [19]. Similarly, in 533 patients with acute coronary syndrome had followed in terms of revascularization and acute heart failure, and it had been concluded that bilirubin was might be associated with a mortality risk at the end of year 2,4. Also many experimental data obtained in animal models strongly supported the protective role of bilirubin [20]. However, clinical studies regarding the prognostic role of total bilirubin in patients with acute myocardial infarction (AMI) in short-term are conflicting. Marginally higher serum total bilirubin level may be a predictor of major cardiac events and cardiovascular death in patients with AMI. Whereas higher baseline bilirubin levels were significantly associated with an increased risk of short-term mortality hazard ratio (HR) 2.35, (95% confidence interval (CI) 1.15-4.77) in the AMI group [21]. When conflicting results were found in the literature, new studies are required.

Therefore in another one meta-analysis the effect of serum total bilirubin level on the risk of atherosclerotic heart diseases were evaluated. A total of 20 studies (323,891 cases) were included in the meta-analysis and identified as coronary artery disease, acute coronary syndrome, stable angina, coronary revascularization, atherosclerotic stroke or transient ischemic attack, and peripheral artery disease (PAD). This meta-analysis showed that bilirubin was significantly negatively associated with cardiovascular mortality, major adverse cardiac events, and AMI prognosis, bilirubin levels. According to this reported, it can be concluded that higher serum bilirubin showed significant negative relationship with cardiovascular disease (HR = 0.83 (95% CI 0.73-0.94) [22]. Later the relationship between serum bilirubin levels and PAD has also been extensively investigated. Increased serum total bilirubin levels were associated with decreased PAD prevalence. Even the combined analysis showed that lower bilirubin levels were significantly associated with PAD (OR = 0.91 (95% CI 0.85-0.98)) [22]. Also in 2008, Perlstein et al. published a retrospective study on more than 7000 adults from the National Health and Nutrition Examination Survey (NHANES). In this study, every 0,1 mg/dL (1,7 µmol/L) increase in serum bilirubin level after adjustment for possible confounding factors was associated with a 6% decrease in the incidence of PAD [23].

In addition to PAD research other studies have shown that bilirubin is associated with intracranial atherosclerosis (ICAS). In a population-based study to investigate the epidemiology and natural history of asymptomatic ICAS in middle age and older adults, after adjusting for all contradictions, bilirubin levels were found to be negatively associated with ICAS, especially in individuals over 60 years of age. This result showed that bilirubin can have a protective effect on ICAS, especially in older individuals [24]. Clinical trials of bilirubin and results are given in Table 1.

**Bilirubin and metabolic disturbances leading to atherosclerosis**

Considering the atheroprotective effects of bilirubin, it is not surprising that the same negative correlations exist for other diseases commonly associated with atherosclerosis. So there are numerous studies showing that bilirubin levels are associated with metabolic syndrome or diabetes. The first report published in this context was about the association of bilirubin with metabolic syndrome between children and adolescents. Furthermore, in diabetic rats, up-regulation of heme oxygenases1 increases serum bilirubin, reduces superoxide anion and endothelial sloughing induced by hyperglycemia [19].This graded relationship was also significantly preserved after the adjustment of other co-variables [25]. Another important study

Table 1:Clinical studies for bilirubin and atherosclerotic heart disases.

| Studies | Participants of study | n | Change of parameters | Results |
|---|---|---|---|---|
| Schwertner H et al. 1994 | Coronary artery disease (CAD) | 900 | %50 decreases in Total bilirubin | %47 increases in CAD severty |
| Vitek L et al. 2002 | With Gilbert Syndrome | 50 | Increase serum bilirubin | %2 decrease of CAD prevalence |
| Novotny L and Vitek L. 2003 (meta-analysis) | With Gilbert Syndrome | | 1.0 µmol/L increases in serum bilirubin | %6.5 decreases in cardiovascular diseases (CVD) |
| Huang ss et al. 2010 | Cardiac X syndrome | 108 | Lowest serum bilirubin | Higher myocardial infarction(MI), ischemic stroke, unstable angına (UAP) |
| Huang FY et al. 2017 | CAD | 3013 | Serum bilirubin | Pozitif correlation with short-term mortality in AMI<br>Negatif correlation with long-term mortality in CAD and UAP |
| Tanak M et al. 2009 | Cardiovasculer Heart disease (CHD) | 637 | Serum bilirubin | Associated with coronary artery calcification (CAC) score |
| Kang Sj et al. 2013 | Non-CAD | 2682 | Higher Serum bilirubin (>1.2mg/dL) | Lower prevalence >%50 stenotic coronary plaque |
| Xu C et al. 2019 | Acute coronary syndrome | 533 | Serum bilirubin | Association with revascularization, acute heart failure and mortaliy |
| Yang L et al. 2019 (meta-analysis) | Atherosclerotik cardiovasculer disease (ASCVD) | 323,891 | Serum total bilirubin<br>Lower serum total bilirubin | Negative association with cardiovasculer mortality, major advers cardiac events and prognosis of AMI<br>Corelation with peripheral artery disease(PAD) |
| Perlstein TS et al. 2008 | NHANES | 7000 | 0.1 mg/dL increases in serum bilirubin | %6 decrease in incidence of PAD |
| Zhong k et al. 2020 | Asymptomatic intracranial atherosclerosis (aICAS) | | Serum bilirubin | Negative association wtih aICAS |
| Lin LY et al. 2009 | Methabolic syndrome(MS) | 7177 | Lower serum bilirubin | Higher prevalence of MS |
| Cheriyath P et al. 2010 | NHANES | 15,867 | Increases in total bilirubin | % 26 decrease in diabetes risk |
| Fu YY et al. 2010 | Hyperbilirubinemic Gunn rats | | Pretreatment with 0.1 mg/dL bilirubin | Decrease cell death and apoptosis in the cell line of rat insulinoma |
| Jıraskova A et l. 2011 | Type 2 diabetes | 700 | 0.1µmol/L increase in serum bilirubin | Reduce the likelihood of developing diabetes |
| Vitek et al. 2002 | Gilbert syndrome | | Increased serum bilirubin | Increased total antioxidant capacity (TAS) |
| Hwang Hj et al. 2011 | Healty people | 2307 | Total and direct bilirubin | Negative association with serum CRP |
| Seung JK et al. 2013 | CAD | 2862 | Higher serum bilirubin (>1.2 mg/dL) | Lower serum CRP |

reported a strong relationship between bilirubin and diabetes. In the study of 15,876 participants from NHANES between 1999 and 2006, total bilirubin increase after age adjustment was associated with a 26% decrease in diabetes risk (or 0.74, 95% CI 0.64 - 0.88) [26].

This relationship has also been supported by studies on animals. Korean researchers reported that hyperbilirubinemic Gunn rats showed significant resistance to developing diabetes after exposure to intraperitoneal streptozosine compared to their normobililubinemic offspring. It has also been found that pretreatment with bilirubin (0.1 mg / dL) in the cell line of a rat insulinoma reduces cell death and apoptosis caused by streptosis and suppresses $H_2O_2$ production [27]. A study published in 2011 covered more than 500 patients with type 2 diabetes mellitus and 200 healthy controls. The findings showed that serum bilirubin levels were low and that the probability of developing diabetes increased every 1,0 µmol/L [28].

Recently reported that higher serum concentrations of bilirubin are associated with a decreased risk of developing CAD and all-cause death in diabetic patients. So serum bilirubin improves the may be risk predictions of cardiovascular and total death in diabetic patients [29]. In conclusion, low serum total bilirubin levels were associated with a significant increase in the risk of diabetes in patients with impaired fasting glucose [27]. In observational studies being included in metabolic syndrome and diabetes, including meta-analyses involving participants almost one million, the negative relationship between serum bilirubin levels and metabolic syndrome with diabetes has been shown once more [30]. Finally, a meta-analysis showing the relationship between bilirubin and atherosclerosis was published. According to the findings obtained in this meta-analysis, higher bilirubin significantly has improved the good prognosis of atherosclerotic cardiovascular diseases [22].

**Gilbert Syndrome and Bilirubin**

Last two decades to investigate the link between bilirubin and reduced risk of cardiovascular disease, the relationship between oxidative stress, inflammation, and markers of vascular dysfunction and bilirubin has been clinically investigated [5]. After all, many beneficial advised linked to moderate elevations of serum bilirubin have been recognized. Thus, Gilbert Syndrome provides invaluable evidence for investigating the anti atherosclerotic effects of bilirubin.

Also the effects of hyperbilirubinemia on decreasing plasma levels of advanced glycosation end products (AGE) that contribute to atherosclerosis have been investigated [31]. As it is known, increased AGE formation in the collagen in the artery wall contributes to the Atherosclerosis process by increasing the rigidity in the vessel structure. In addition, AGE-bound collagen found in the vascular structure accelerates the formation of plaque by binding to LDL. In one of the clinical trials related to Gilbert Syndrome, serum levels AGE were significantly lower than the in these hyperbilirubinemic individuals [31]. In another study involving subjects with Gilbert Syndrome, with kidney diseases were significantly lower compared with the control group [32].

The relationship between bilirubin and total antioxidant capacity (TAS) has been reported in some studies. While, Vitek et al published in 2002 in a study involving Gilbert cases, and the control group. They found that TAS differed significantly between these groups. TAS was significantly higher in patients with Gilbert Syndrome compared with control cases. As showed in the other in vitro experiment, serum levels of TAS was might be associated with increased bilirubin concentration [12].

**Chronic inflammation and Bilurubin**

The elevation of bilirubin concentration in plasma is well known as a marker of hemolytic conditions, liver damage or bile-duct impairment. Endothelial activation and recruitment of inflammatory cells are two pivotal steps in the development of atherosclerotic lesions [2,3]. Especially, also the production of bilirubin in peripheral tissues has been proposed to be protective the anti-inflammatory effect of bilirubin was already observed in hyperbilirubinemic rats. Also in vitro studies demonstrated that bilirubin prevents TNFα-induced leukocyte adhesion to endothelial cells by reducing the expression of pro inflammatory molecules [2-4].

There are also studies focusing on the relationship between C-reactive protein (CRP) and bilirubin, a marker that reflects chronic vascular inflammation. For example a cross-sectional study of 2307 healthy Korean adults found that total and direct bilirubin elevations were associated with low serum CRP levels. After adjusting age, body mass index, hypertension, diabetes, hypercholesterolemia, cardiovascular disease, aspirin, smoking, alcohol and regular exercise, the negative association of CRP with both total and direct bilirubin was maintained. Thus it is hypothesized that low serum CRP levels may be due to the antioxidant and antiinflammatory effects of bilirubin metabolism [33]. In another study (n=2862), those with a high serum bilirubin level (>1,2 mg/dL) had a lower CRP level than those with a low serum bilirubin level (<1,2 mg/dL) (34). Slight increases in plasma total bilirubin concentrations (1.53 ± 0.48 mg/dl) have been reported to preserve flowmediated vasodilation compared to subjects with low levels of plasma bilirubin (0.40 ± 0.08 mg/dl(10,35). Also recently repoted that the serum total bilirubin level was found to be $0.41 \pm 0.21$ ng/dL in the severe erectile dysfunction, $0.43 \pm 0.19$ ng/dL in the moderate erectile dysfunction, and $0.48 \pm 0.11$ ng/dL in the mild erectile dysfunction groups. Also the prevalence of multiple sclerosis was 6.6 ± 1.2% in the group with the lowest bilirubin concentration, while bilirubin concentration was 2.1 ± 1.9% in the highest bilirubin concentration group [36].

Bilirubin is able to protect against atherosclerotic diseases by means of different metabolic pathways? We can only answer this question by looking at studies related to Hem metabolism.

**Heme and Atherosclerosis**

Heme, an amphipathic iron-protoporphyrin complex, is one of the most important prosthetic groups on Earth, which serves as an oxygen transporter and participates in various oxido/reductive processes in aerobic and anaerobic cell metabolism. However, free heme released from the safe sanctuary area of heme proteins triggers a number of adverse events. In last two decades, several reports revealed the detrimental role of heme in neuronal damage and the potential role of heme in health and disease. Heme, due to its amphipathic nature, shows high affinity towards biological membranes, sensitizing them towards reactive oxygen species and leading to the oxidative damage of membrane lipids, cell lysis, genomic, and mitochondrial DNA damage [34]. For these reason recently administration of FDA-approved hexyl 5-aminolevulinate hydrochloride has begun to be used in clinical trials to produce anti-inflammatory carbon monoxide and bilirubin in atherosclerosis [37].

**Bilirubin and Heme Oxygenases**

Heme oxygenase is the first, rate-limiting enzyme in heme degradation pathway, with two major isoforms of heme oxygenase identified. First Heme oxygenase-1 is inducible whereas, expressed only under oxidative stress or when heme oxygenase-2 [38,39].

There are several experimental conditions where hem oxygenases-1 provides defense for cells and tissues. Heme oxygenases-1 catalyzes the opening of the prothoporphyrinic ring of heme, generating biliverdin, free iron and carbon monooxide (CO) (Figure 3). Moreover, biliverdin is considered to be an endogenous antioxidant in several clinical conditions [37]. CO has anti-apoptotic and antiinflammatory activities. While CO is an anti-inflammatory and anti-apoptotic gas molecule. The scientific importance of CO is increasing, since quite a lot of work proves its anti-inflammatory effects. Considering the protective effect of Heme oxygenases, it is a logical explanation that end-products of heme degradation, bilirubin, biliverdin, and CO are responsible for the beneficial action. The generation of free iron is potentially highly toxic but, under physiological conditions, a parallel induction of the heavy chain of ferritin, and the activation of membrane Fe-ATPase transporters occur. To eliminate the redox active free iron, cells rapidly express ferritin. The antioxidant character of ferritin depends on its ferroxidase activity and iron sequestering capability [39]. The possible mechanisms of underlying the relationship between high bilirubin and decreased atherosclerosis as monitored: bilirubin could effectively block the generation of cellular reactive oxygen species and intercellular adhesion molecule. High serum bilirubin levels resists for myeloperoxidase-induced oxidation and to prevent the formation of atherosclerosis; and higher bilirubin also has related for an anti-inflammatory effect on atherosclerotic process [37] (Figure 4). It has clearly been shown that unconjugated bilirubin mimics the hypolipidemic activity of fenofibrate. These data show that, in the vessel wall, the activity of bilirubin on lipid metabolism is complex and merits further investigation. As recently reviewed, bilirubin prevent platelets aggregation due its ability to interfere with the surface expression of adhesion molecules and its antioxidant activity, thereby supporting a role played in the prevention of hypercoagulability and thrombosis [39].

Figure 3: Heme oxygenase and products.

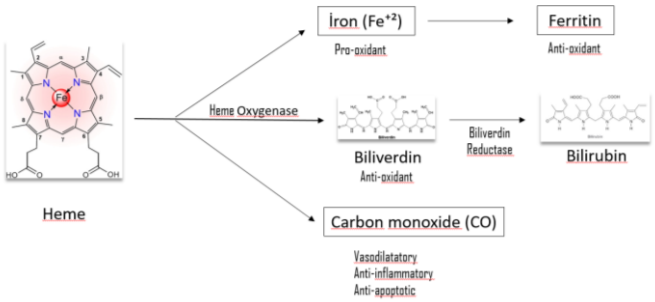
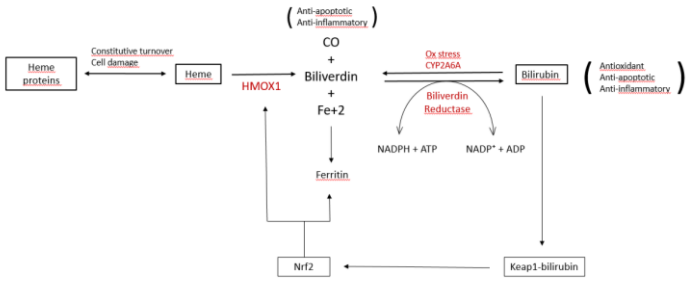



Figure 4: Enzymatic reactions of bilirubin generation and cytoprotection




HMOX1:Heme oxygenases1, CO:Carbon monoxide, Nrf2:Nuclear factor erythroid-2 related factor, Keap1:Kelch ECH-associating protein 1.

Heme oxygenases1 catalyzes the degradation of heme groups to CO, Fe2+ and biliverdin, the latter subsequently converted to bilirubin by biliverdin reductase. By reaction with oxidant species, bilirubin is oxidized back to biliverdin, amplifying the antioxidant effect. Bilirubin and CO exert anti-apoptotic and anti-inflammatory activity. Fe2+ is quenched by the heavy chain of ferritin, and further released to form heme. A positive feedback of cytoprotection can be generated by the ability of bilirubin to bind nucleophiles such as thiol reactive cysteines on Keap1, favoring Nrf2-dependent HMOX1 gene transcription.

**Conclusions**

In this review, several experimental and clinical studies investigating the relationship between atherosclerotic diseases and bilirubin and the effects of bilirubin on the atherosclerotic process were examined. Although different results are obtained in some studies, the protective properties of bilirubin are observed at every stage of the Atherosclerosis process. Clinical studies examining the relationship between Bilirubin and atherosclerotic diseases have suggested that low serum bilirubin concentrations are associated with increased risk of.

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