Divergent impact of gender in advancement of liver injuries, diseases, and carcinogenesis

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1. ABSTRACT

Several investigations have revealed that liver diseases exhibit gender biases, but identifying the root causes of such biases has been challenging. Evidence of gender differences in liver function is present from the early stage of embryonic development. The differences in access to care and treatment as well as diagnostic deliberation may affect gender-specific differences in liver disease progression. Apart from the pathogenesis, xenobiotic metabolism, immune responses, gene expressions, mitochondrial function, lipid composition, and enzyme activities also differ in this sexually dimorphic organ. Differences in a social environment and lifestyle of men and women may also be involved in the basic mechanisms underlying the sex-associated differences and protective or aggravating effects of sex hormones during viral infections, alcoholic and non-alcoholic chronic and/or acute mode of liver injuries, carcinogenesis, autoimmune responses, and liver
transplantation outcome. We summarized here the recent findings regarding the influence of sex hormones on immune responses underlying the pathology of the liver diseases in humans and animal models.

2. INTRODUCTION

As a major metabolic organ, liver is continuously exposed to a large number of antigenic loads that includes pathogens, toxins, xenobiotics, and dietary antigens. Several pathological conditions associated with liver include alcoholic liver disease (ALD), non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), autoimmune hepatitis, cirrhosis, primary biliary cirrhosis, sclerosing cholangitis, acute liver failure and carcinoma. Additionally, viral hepatitis B and C (HBV, HCV) can cause chronic infection, which ultimately can lead to cirrhosis and hepatocellular carcinoma (HCC) (1, 2). Regarding gender biases, several reports have revealed clear evidence of sex differences in the pathophysiology of liver diseases. Previous studies have shown that the prevalence of hepatitis is higher in male population as compared to female population (3). One possible reason behind this difference could be better management of infection-induced oxidative stress by females. Female sex hormones such as, estrogen, act as an antioxidant and can have cytoprotective roles against liver pathology. Interestingly, women show higher susceptibility to alcohol-induced liver injury, but are much less vulnerable to liver carcinoma, primary sclerosis, cholangitis, and viral hepatitis than men (4). Similarly, women with HCV infection develop decompensated cirrhosis at much slower rate than men. Moreover, women are 10 times more likely to have primary biliary cirrhosis than men and 4 times as likely to have autoimmune hepatitis (5, 6).

The interactions between sex hormones and the immune system are known to render one particular sex more susceptible to pathogen-associated molecular pattern (PAMPs) and damage-associated molecular patterns (DAMPs) (7) along with genetic (chromosome) differences (8). The role of X and Y chromosome heterochromatin in regulating epigenetic states of autosomes has highlighted the unconventional mechanisms of gene regulation and can serve as an important component of gender biases (9, 10). Studies have linked immunomodulatory functions of sex steroids to higher infection rates in males with down-regulated testosterone. A recent study has demonstrated that the immunomodulatory role of X-chromosome-linked micro RNAs might be responsible for better immunity in females (11). Moreover, despite having a strong immune system, females are often at higher risk of autoimmune disorders. Furthermore, several studies have shown that certain gut microbial species, which are predominant in males, can have a protective role against type 1 diabetes. Molecular interaction between microbiota and sex hormones might play a role in gender biases (12).

Altogether, a better understanding of the basic mechanisms underlying gender-associated differences in severe pathogenic conditions such as hepatic fibrogenesis and carcinogenesis, may open up new opportunities for the prevention and treatment of lethal diseases as well as can broaden our knowledge towards gender medicine. This review highlights current knowledge and outcome of the gender-associated clinical observations of liver diseases in relation to pathogenesis as well as the progression of the chronic and acute mode of injuries and tumorigenesis.

3. VIRAL HEPATITIS: GENDER DISCREPANCY

3.1. HBV Occurrence: Predominant in male

HBV can cause severe chronic and acute hepatitis, liver diseases, cirrhosis, and HCC in humans (13, 14). Chronic HBV infection remains a global health problem, affecting an estimated 240 million individuals (14). The role of HBV has been extensively studied, but the mechanisms of chronicity and pathogenicity in the liver and extra hepatic tissues as well as the regulation of HBV replication and gene expression are still inadequately understood (15). HBV is generally transmitted by prenatal, parenteral, and sexual routes. For prenatal transmission, the predominant route is through mother to infant; however, father-to-child transmission also plays an important role in the prevalence of hepatitis B (16). The transmission of HBV infection to children from their carrier fathers could be either horizontal through intimate postnatal contact, or vertical via the male germ line. The nucleoside analogs (NAs) and Interferon (IFN) are two major anti-HBV drugs in clinical practice. NAs hinder replication of the HBV DNA replacing the nucleoside during HBV polymerase extension, resulting in termination of chain extension. These reduce the amount of HBV in the blood to achieve therapeutic improvement. However, NAs greatly inhibits viral replication and relieves inflammation but does not eliminate the virus completely (17). NAs almost always produce drug resistance and deterioration after discontinuation of therapy. Another drug IFN acts as an immune regulator by inducing host cytokines to inhibit multiple aspects HBV replication. The European Association for the Study of the Liver (EASL) (18) has designated that IFN therapy is the favored treatment option for HBeAg (hepatitis B viral protein)-positive patients who attain stable HBeAg seroconversion and for HBeAg-negative patients who attain sustained response after therapy. But still current treatments for chronic hepatitis B (CHB) with INF are limited by the low rates of sustained response, side effects, and the emergence of drug resistance (19).
The key barrier against curing CHB is the difficulties to eliminate or inactivate covalently closed circular DNA (cccDNA) (20). Previous studies have shown that the rate of chronic infection by HBV is higher in men due to various factors mainly sex hormones (21). The frequency of hepatic infection in males is ~ 80% and in females ~ 20%, with a male to female ratio of 4:1(22). However, it is still uncertain if men are exposed to more viruses, or if men have a less effective immune response.

The low frequency of chronic infection in females may be due to involvement of genes located on the X chromosome. This hypothesis is supported by the occurrence of hepatic autoimmune diseases in females. In fact, the HBV does not significantly influence the fertility, and the occurrence of HBV infection during pregnancy does not increase the morbidity and mortality rate of both the mother and fetus (23). On the contrary, during normal pregnancy, elevated levels of corticosteroid hormones and estrogens are known to trigger HBV infections with high alanine aminotransferase (ALT) levels (24, 25). Few studies have verified that the increased production of pro-inflammatory cytokines in CHB (26, 27) may contribute to the development of complications in pre-term delivery, pre-delivery hemorrhages as well as gestational diabetes (28). Moreover, higher frequencies of gestational hypertension, detachment of the placenta, and peripartum hemorrhages were found in women with cirrhosis developed by infection of HBV (29). So far, there are no suitable therapeutic treatments to prevent viral transmission. The maternal transmission of HBV may take place through placental (30), breast milk or by the contact with maternal cutaneous lesions. The pre-delivery administration of immunoglobulins has shown discordant results (31, 32). The administration of immunoglobulins and anti-HBV vaccine within 12 h of birth have shown to reduce the frequency of HBV transmission from > 90% to 26% (33, 34). It is known that the Lamivudine treatment before pregnancy or in early stage of pregnancy of HBV infected women with or without co-infection with hepatitis C virus (HCV), human immunodeficiency virus, cytomegalovirus, or other viruses blocks mother-to-infant transmission of HBV without any adverse effects. Additionally, no effects on fertilization, embryonic development, or congenital abnormalities in infants are demonstrated (35). As per case reports, the treatment with triple therapy of Lamivudine, IFN-β, and prednisolone for acute or CHB exacerbation are safe (36). Other meta-analysis have predicted that the Telbivudine used in the final stage of pregnancy is effective in preventing or reducing the perinatal transmission of HBV without unfavorable effects (37). However, several ongoing projects are still investigating the use of antiviral medicines in mothers with high HBV DNA levels. Recently, Food and Drug Administration (FDA) approved Telbivudine and Tenofovir as antiviral drugs with no risk as per their pregnancy regulations (38).

3.2. HCV Occurrence: Protective role of estrogen in female

An estimated 150 million people worldwide are infected with HCV, another major aetiological cause of chronic liver infection. Although the infection of RNA virus HCV causes significant induction of the host immune response, the majority of HCV infection develops into fibrosis. Subsequently, chronic hepatitis C (CHC) evolves into cirrhosis and HCC frequently and therefore, exerts serious impact on public health (39). Several studies have demonstrated that women have less altered hepatic biochemical parameters and lower rates of fibrosis progression induced by HCV infection than men (40). Reports have also shown that the ability to spontaneously clear HCV infection is higher in women than in men (41). Furthermore, during early infection, HCV RNA levels are higher in men than in women (42). Similarly, cirrhotic progression rarely occurs in pre-menopausal women (43) probably due to the protective role of estrogen and estrogen-mediated signaling pathway. The estrogen favors the cleavage of the tight junction protein, named occludin, one of the proteins that HCV uses to gain access to the hepatocyte (44). Estrogen binds to the specific receptors expressed in immune cells, thereby influencing adaptive and innate immune responses (45) by triggering the JAK/STAT pathway (46). In agreement with these studies, there is evidence that menopause is associated with an accelerated rate of fibrotic progression and that hormone replacement therapy may minimize this effect (42).

The transmission of HCV generally takes place by parental routes, such as contact with infected blood or contaminated materials and intravenous drugs injection with contaminated syringes. HCV can also be transmitted sexually from a HCV-positive partner (47). The occurrence of HCV infection during pregnancy is 1%–2% in the United States and Europe. However, the rate is quite high in some developing countries (48). In fact, the mother-to-child transmission frequency of HCV is approximately 5%–10% (49). But the amniocentesis, the extended breaking of the membranes, and an elevated viral load in the mother can increase the risk of maternal to fetal transmission of HCV. Few case studies have shown that high levels of ALT, a major biomarker of liver injury, before pregnancies are connected with higher maternal to fetal transmission of HCV. Thus, a liver injury in the mother can be a potential risk factor for HCV transmission (50). Furthermore, the co-infection with HIV virus can also increase the likelihood of vertical HCV transmission by 90% (51). Regarding therapy, PEG-IFNa, ribavirin, telaprevir, and boceprevir are considered as standard treatment regimen for chronic HCV infection (52). Research has
found that the sustained virological response (SVR) rate is significantly higher in women than in men, and fertile women with normal genotypes have a 100% chance of obtaining SVR.

Several studies promise in identifying immune-metabolic deregulation in the context of HCV occurrences (53). During HCV infection a robust innate immune response is involved with the induction of several interferon-stimulated genes (ISGs) and mediated by production of inflammatory and antiviral cytokines by macrophages, natural killer (NK) cells and neutrophils, which release perforin, granzyme B, interferon-gamma (IFN-γ) and tumor necrosis factor β. During adaptive immune response both CD4+ and CD8+ T cells have been shown to play major roles. CD8+ T cells inhibit HCV replication by cytolytic and non-cytolytic effector mechanisms that are highly dependent on CD4+ T cell activation (54). Unbalanced T-helper CD4+ cells subsets in the liver causes the hepatic inflammation and subsequent liver fibrosis. An increase energy requirement especially with glucose uptake and glycolysis is essential to sustain the effector functions of T cells involving PI3K/Akt, mTORC, HIF1α, p70S6K and Bcl-6 signaling pathways (55). These context of immune-metabolic insights may improve the gender specific therapeutic manipulation against inflammatory conditions during viral infections as well as autoimmune disorders and metabolic syndromes.

4. GENDER BIASES IN HEPATIC FIBROSIS: ROLE OF miRNA

Hepatic fibrosis is a fibrous scarring of the liver where excessive collagens build up with time and degree of persistent liver injury. In this scenario, the excess collagens are deposited in the injured areas instead of damaged hepatocytes. With respect to the liver, the sinusoidal pericytes known as the hepatic stellate cells (HSCs), which account for 5%–8% of the liver cells, contribute to the fibrogenesis response of the liver during chronic disease states. These activated HSCs engulf the apoptotic bodies resulting from hepatocyte death and produce profibrogenic cytokines such as transforming growth factor-β 1 (TGF-β) and type I collagen. Activation of HSCs and their transdifferentiation into myofibroblasts with the secretion of extracellular matrix proteins play a critical role in vigorous fibrogenesis (56). The damaged hepatocytes resulted from continuous liver injury release the nucleotides ATP and UTP which can in turn bind to the purinergic G protein coupled P2Y2 receptors to activate stellate cells during fibrogenesis (56). It is well documented that the progression of hepatic fibrosis in chronic hepatitis B and C is slower in females than in males (57–60). In case of the chronic infection, females produce antibodies against hepatitis B surface antigen (HBsAg) and hepatitis B viral protein (HBeAg) at higher frequencies than males (61, 62) especially before menopause. Similarly, it is also known that the clearance rate of HCV RNA from the blood is higher in women than men (63). Because of the higher clearance rate, female asymptomatic carriers of HCV with persistent normal ALT have good prognosis with low risk of progression of hepatic fibrosis to the end-stage cirrhosis and its complications such as HCC (64). Due to the effect of sex hormone, menopause is linked with accelerated development of hepatic fibrosis and the risk of HCC is inversely related to the normal age of menopause (65). Previous studies using fibrosis-inducing reagents such as carbon tetrachloride (CCl4) have also shown a clear effect of gender differences on fibrotic responses in animals. In general, female animals are known to be more resistant to CCl₄-induced fibrosis than male animals. A number of research groups have identified the protective effect of estradiol in inhibiting hepatic fibrosis in CCl₄-induced animal models (66–68). Interestingly, estradiol is also known to reduce the mRNA levels of type I and III procollagens and the tissue inhibitor of metalloproteinase-1 (66). In a sterilized female animal model, the estradiol substitution shows fibro-suppressive effect. The protective role of estradiol against liver fibrosis is clearly related to its inhibitory effect on hepatic stellate cell (HSC) activation (67). Moreover, several reports have demonstrated that microRNAs play significant role in the progression of liver fibrogenesis through the activation of HSCs (69). The over-expression of miR-16 and miR-15b has shown to inhibit the proliferation of HSCs and to induce apoptosis through the down-regulation of anti-apoptotic protein Bcl-2 and the activation of effector caspases (70). Similarly, the overexpression of miR-29b in murine HSCs has shown to decrease collagen deposition (71) and miR-29 family have shown a significantly down-regulated profile in CCl₄ induced liver fibrosis model as well as in mice that underwent bile duct ligation (BDL). Most interestingly, the data from Zhang et al. have demonstrated that the levels of miR-29a and miR-29b were significantly decreased in the livers of male, but not in female mice following a 4-week CCl₄ treatment (72). The down-regulation of miR-29a and miR-29b in male mice was correlated with the accelerated progression of liver fibrosis by increasing expressions α-smooth muscle actin (α-SMA), TGF-β1, and collagens as compare to female mice. The estradiol that enhanced the expression of miR-29a and miR-29b in cultured murine hepatoma was maintained at a higher level in female mice than in male mice. Furthermore, the recombinant adenovirus expressing miR-29a and miR-29b markedly attenuated the expression of collagen I and α-SMA in the mouse liver confirming the protective role of estradiol induced miR-29s expression in CCl₄-induced hepatic fibrosis in animal model (72). Considering all these benefits, miR-29a and miR-29b are currently implicated as the key collagen regulators during tissue fibrosis (71, 73). Recently Kwekel et al. carried out the genome-wide characterization of liver miRNA expression profiles in male and female during life span using rat model and
predicted functional pathways that may underlie gender specific susceptibilities to liver toxicity and disease (74).

5. GENDER VARIATION IN HEPATOCellular CARCINOMA

Hepatocellular carcinoma (HCC) is the 6th most prevalent cancer and the 3rd most frequent cause of cancer-related death that accounts for approximately 6% of all human neoplasms (75). In the context of gender variation, it is 5th most common malignancy in men and the 9th most common in women estimated over 0.5 million cases occur every year worldwide. According to the literature, men are 2–3 times more susceptible to HCC as compared to women. However, controversial reports regarding the effect of gender differences on patient survival and prognosis do exist. However, very few studies have evaluated the clinico-pathologic characteristics of patients and their impact on survival with specific reference to gender in a large sample cohort. HCC occurs more often in males with chronic liver disease which in turn leads to a hyper estrogenic state that has been implicated in the pathogenesis of HCC (76–79). These distinct incidences have been described by the link between estrogen and inflammation-induced carcinogenesis (Figure 1) (80, 81). In this direction, animal studies have demonstrated that the males have shorter survival time and larger tumor volume than females because of estrogen induced reduction of tumor malignancies (81). The work by Wang et al has described that the ovariectomized animals treated with estrogen showed a significant decrease in HCC progression in comparison with animals that underwent ovariectomy alone (82). In this context, several reports have predicted that the higher risk of HCC among men is due to higher frequency...
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of alcoholic cirrhosis in general but significant studies have also suggested a carcinogenic effect of testosterone. Reports have suggested that the testosterone can stimulate mitogenic actions through Transforming Growth Factor alpha (TGFα) that could in turn increase hepatocyte proliferation as well as HCC (83). Growing interest in this field has guided efforts to resolve the mechanism behind the gender differences in HCC. Several studies have found that both estrogen and androgen have significant effect in controlling the replication rate of hepatic cells (84, 85) and thereby have an effect on inducing or at least endorsing the progression of HCC. Based on several evidence, a number of clinical trials have been carried out using antiandrogen and antiestrogen therapies. However, these therapies have not shown a clinically significant effect on the progression of the disease once the tumor is developed (76, 79, 84). On a different note, it has been reported that the DNA synthesis is higher in male than in female cirrhotic liver and the higher rate of cell turnover has been predicated to be a contributing factor to the gender biases in HCC (86, 87). In a coherent study, Keng et al have approached the Sleeping Beauty (SB) transposon system to illuminate the candidate oncogenic drivers of HCC in a forward genetics screening approach. They have analyzed the liver nodules isolated from both male and female mice with different genetic background in order to elucidate the gender bias nature of HCC. In this study, they have found that the epidermal growth factor receptor (EGFR) insertions were enriched in male mice but infrequent in female mice. They have also confirmed the findings with human data showing more frequent polysomy of chromosome 7 (locus of EGFR gene) in males than females, the phenomenon associated with EGFR overexpression (88). Moreover, other experiments with induced tumor model have demonstrated that the male mice are more sensitive than female mice to diethylnitrosamine (DEN) induced HCC because of the hormonal factors (89). However, the effect of gender differences in connection with Bcl-2 family proteins and caspase activation during carcinogenesis is a big challenge to understand the basic biology of tumor progression (90, 91). In another perspective, the number of death cells along with the protein levels of anti-apoptotic Bcl-2 (female< male), pro-apoptotic Bax (female< male), and Bad (female< male) have shown differences among male and female in the central division of the medical preoptic nucleus (MPNc) (92). Thus, the patho-physiology of the tissue degeneration of HCC in context of gender is itself under positive surveillance.

6. ACUTE LIVER FAILURE: PREVALENCE AMONG FEMALES

Acute liver failure (ALF) is a broad term that includes both fulminant hepatic failure and sub-fulminant hepatic failure (or late-onset hepatic failure). Fulminant hepatic failure is usually used to define the progress of encephalopathy within 8 weeks of the onset of the symptoms in a patient with a previously healthy liver. Sub-fulminant hepatic failure is earmarked for the patients with liver disease for up to 26 weeks before the development of hepatic encephalopathy. Furthermore, the acute liver injury develops more rapidly and more extensively in women than in men even for a lesser quantity of alcohol consumption or drug overdose (93). Different patterns of drug-induced liver damage exist between males and females. By excluding the behavioral or dosing differences, there are three main hypotheses regarding the mechanism behind these differences, including: (a) different pharmacokinetics between females and males; (b) gender specific hormonal effects or interaction with signaling molecules that may affect drug safety; and (c) differences in aberrant immune response that targets the liver following the drug exposure that can result in adverse drug effect (93). These gender-specific differences have also an impact on subsequent toxicity including differences in relative amount of circulating drug-binding proteins, gastrointestinal blood flow, gastric acid secretion, relative proportions of adipose and muscular tissues, renal blood flow, and gender-based expression of cytochrome P450 isozymes as well as physiologic and hormonal changes during the menstrual cycle during pregnancy and after menopause.

The liver is a highly regenerative organ. However, during chronic liver injury due to aberrant regeneration with increased expression of TIMPs and subsequent of the scar-resolving function of MMPs, the liver undergoes scarring by inhibiting the proliferation of mature epithelial cells with downregulation of CXCR7 and upregulation of CXCR4. As a result, large number of hepatocytes undergo senescent stage frequently (94). However, the liver regenerates efficiently immediately after the acute injury (95, 96). This process requires a sudden coordinated response of immune cells including T cells, macrophages, and eosinophils, mobilization of liver growth factors, matrix remodelling, and a rapid but tightly controlled abundance of epithelial cells to sustain the cell cycle and proliferation of hepatocytes (94, 97, 98). During acute liver insult or in acute and chronic liver failure (ACLF) (99) due to alcohol or drug consumption, the liver microenvironments are exposed to instant high levels of proinflammatory cytokines such as TNF-α, IL-1α, and INF-γ caused by host derived (‘DAMPs’) or pathogen derived (‘PAMPs’) danger signals (e.g. LPS). These cytokines, besides further potentiating the inflammatory response, activate both survival and cell death pathways depending on the level of exposure and cellular context.
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7. ALCOHOLIC LIVER INJURY AND TOXICITY; GENDER SPECIFIC VULNERABILITIES

7. 1. Influences of alcohol in metabolic and immune dysfunction

Ethanol hepatotoxicity is associated with the metabolism of ethanol by alcohol dehydrogenase (ADH) and hepatic microsomal ethanol oxidizing system (MEOS) to acetaldehyde, which is subsequently converted by aldehyde dehydrogenase (ALDH) to acetate (100). The cytochrome P450 2E1 (CYP2E1) is the key enzyme of the MEOS and is involved in the oxygenation of ethanol and fatty acids. Although ethanol is mostly oxidized by ADH, the CYP2E1 resumes more after chronic consumption of ethanol due to its high NADPH oxidase activity. NADPH/NADH oxidase is a primary source of reactive oxygen species (ROS) production in stellate cells (HSCs) in the space of Disse within the liver. A Person with prolonged heavy drinking habit develops alcoholic liver disease (ALD). ALD has different pathological stages or forms such as early fatty liver (hepatic steatosis), alcoholic hepatitis (AH), alcoholic steatohepatitis (ASH), and cirrhosis though there are considerable overlaps between these conditions. The occurrence of ALD increases in a dose-dependent manner proportionally to the cumulative alcoholic intake. The quantity of alcohol requires inducing hepatitis or cirrhosis varies among individuals. However, as little as 40g per day for 10 years is related with an increased incidence of cirrhosis. The gender specific characterization of excessive alcohol intake is measured as two standard drinks (20 g ethanol) daily for men and one standard drink (10g ethanol) daily for women. In general, men have a greater opportunity to drinking alcohol. However, the habit of alcohol consumption is increasing among women owing to a reduced social disgrace involved in drinking and to the availability of the alcohol in the supermarket. Despite the higher prevalence of alcoholism in men, chronic alcohol intake induces more rapid and more severe liver injury in women. With an equivalent dose of alcohol, females have higher blood ethanol levels than males (101).

Different studies have provided several clarifications in this context: (a) In terms of physical appearance, females are generally smaller than males. Thus, the same dose of alcohol leads to higher blood alcohol levels for females. Similarly, the female body water content is lesser than the male per kg of body weight. Therefore, an equivalent dose of ethanol is distributed in a lesser volume of water in females than in males leading to higher concentration of ethanol in female blood (102). In this reason, stomach faces higher blood alcohol level in the females than the males. (b) Moreover, the stomach acts as a barrier against the absorption of alcohol into the body by retaining and breaking down the alcohol (101) by the action of gastric ADH. The Gastric ADH activity is lower in females than in males. For a given alcohol dose, male ADH level is two times higher than female level and therefore the female blood alcohol levels are higher than those of males. These gender differences in metabolism of alcohol appear to hold for younger adults than to older adults. ADH activity decreases with age, particularly for males, leading to similar blood alcohol concentrations in older males and females or even higher concentrations in older males than older females (101). (c) Hepatic Tumor necrotic factor-α (TNF-α) level, which induces apoptosis, are significantly elevated in female than in male livers. Hepatic IL-6 production, which promotes hepatocyte proliferation, is induced by ethanol only in males but not in female animals. This observed difference in cytokine response may contribute to the enhanced sensitivity of female livers to ethanol-induced injury (103). Alternatively, this manifestation can be due to alteration of the immune system with sex steroids that can regulate gene expression through the steroid-responsive elements, alter antigen presentation through its effects on the human leukocyte antigen genes, and alter the cytokine environment (104, 105). (d) Experiments in rats have also suggested that the higher endotoxin levels and increased gut permeability to endotoxin may contribute to the more severe liver injury in females (106–108). (e) Increased metabolism of ethanol and fatty acid through the CYP2E1 pathway can produce excessive amount of ROS which subsequently can cause oxidative stress induced lipid peroxidation and membrane damage ultimately leading to hepatic cell death. This signal activates inflammatory cells as well as neutrophils, macrophages, kupffer cells, and hepatic stellate cells (HSCs). Like residential macrophages of the liver, the bone marrow derived monocytes can also be stimulated by endotoxin induced proinflammatory cytokines and ROS. The endotoxin stimulated monocytes in males produce more TNF-α as compared to females (109). Previous studies using animal model have demonstrated that the stimulation of the kupffer cells by estrogen can increase the overall sensitivity to endotoxin after ethanol consumption (108). Studies have shown that adding estrogen after ethanol ingestion can enhance TNF-α production in kupffer cells via elevation of the blood endotoxin level and hepatic endotoxin receptor (CD14) expression which in turn results in an increased inflammatory activity in the liver (Figure 2) (110). Another study using cultured HSCs have shown that the estradiol treatment reduces ROS generation through the NADPH oxidase system as well as attenuates TGF-β1 expression, cell activation, and MAPK pathways. On the other hand, treatment with progesterone has shown to increase the ROS generation, TGFβ1 expression, and stellate cell proliferation (111). Moreover, the administration of ethanol in female rats has shown to induce the hepatic activity of CYP2E1. Similarly, the administration of
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- Anti-estrogen has exhibited a reduction in the ethanol-induced CYP2E1 activity (112). Since the activity of cytochrome P-450 (CYP) isoenzyme is regulated by circulating growth hormone, distinct expression pattern of hepatic CYP isoenzymes are observer between females and males due to gender differences in growth hormone secretion profiles (113).

- TLRs response during alcoholic liver injury

Toll-like receptors (TLRs) are the innate pattern recognition receptors for a wide variety of PAMPs such as microbial components, endogenous molecules, and danger signals (114) with DAMSs. Principally, 10 receptors from the TLR family are expressed by granulocytes and the cells of myeloid lineage. In the liver, TLRs are expressed in many cells including Kupffer cells, endothelial cells, dendritic cells, biliary epithelial cells, HSCs, and hepatocytes. The expression of the TLRs and their level of receptiveness on these different cells normally are adjusted to support appropriate reactions to immune challenges and to prevent the misplaced and potentially damaging responses (115, 116). The exposure to alcohol modulates the well organised TLR network by intensifying the TLR responses to external and internal triggers such as PAMPs and DAMPs (117, 118). As per literatures, the lipopolysaccharides (LPS) acting via TLR4 appears to be the most key interaction in the pathogenesis of alcoholic hepatitis. Mice with non-functional mutant TLR4 are protected from alcoholic liver injury (119) whereas the deficiency in inflammatory Interleukin 1 (IL-1) receptor associated kinase (IRAK)-M create more severe alcohol injury (120). Altogether, TLR4 plays an important role in alcohol-induced inflammation by activating two distinct signaling pathways namely (i) the myeloid differentiation primary response gene 88 (MyD88) dependent pathway (ii) the MyD88-independent pathway (116, 117, 120, 121). TLR4-mediated MyD88-dependent signaling triggers the expression of nuclear factor kappa B (NF-κB), a central transcriptional regulator of immune responses and pro-inflammatory pathways, promotes the mitogen activated protein kinase (MAPK) pathway to induce the production of cytokines including TNF-α. In contrast, the MyD88-independent pathway proceeds via a different major adaptor protein called TIR domain-containing adapter-inducing interferon-β (TRIF) and results in the production of interferon regulatory factor 3 (IRF3), type 1 interferons (IFNs), and pro-inflammatory pathways.

Figure 2. Influences of Sex-hormone on Immune dysfunction during alcoholic liver injury. Alcohol and its production of acetaldehyde adducts cause hepatocytes death by oxidative stress, production of reactive oxygen species, caspase 1, IL1 mediated inflammation. Alcohol ingesting leads to intestinal bacterial overgrowth and gut dysbiosis. Disrupted epithelial tight junctions allows the escape of PAMPs into the portal circulation Following chronic alcohol ingestion, endotoxin, also called lipopolysaccharide (LPS), released from intestinal gram-negative bacteria moves from gastrointestinal tract (gut) into the liver via the portal bloodstream Activation of Kupffer cells by gut-derived endotoxin plays a pivotal role in alcoholic liver injury. Estrogen triggers liver Kupffer cells activation. LPS is recognized by the Toll-like receptor TLR4 complex on Kupffer cells in the liver, leading to production of proinflammatory cytokines, particularly tumor necrosis factor TNF-α, and resulting in injury to liver cells (hepatocytes). Endothelial cells reacts with hypoxic inducing factor, Hif1 and initiate inflammations. Hepatic stellate cells (HSCs) activate with production of α-smooth muscle actin (αSMA) and leads towards fibrogenesis. Meantime the systemic circulation of TNF-α and other proinflammatory cytokines causes decreased of gut barrier integrity and increase of bacterial abnormal growth. ADH; Alcohol dehydrogenases. P2Y2; Gq/phospholipase C-linked human receptor.
cytokines (122). The rodent models of alcohol liver disease have confirmed that the TLR4–TRIF signaling plays an essential role in alcohol-induced activation of TLR4 in resident macrophages and the kupffer cells (123). Moreover, TLR4 signaling in both immune cells (i.e., Kupffer cells) and non-immune cells involved in tissue repair (i.e., HSCs) is required for the development of alcoholic hepatitis and fibrosis (124).

Most interestingly, recent studies have focused on the hormonal impacts on the function of immune subsets with high TLR expression. The levels of estrogen receptor expression within the liver differ between the sexes and the estrogen appears to activate liver Kupffer cells (125) with increased inflammation and necrosis that can be counteracted by treating female rats with an antiestrogen (112). The increased intestinal permeability serves as an important factor in intrahepatic cholestasis of pregnancy and is also relevant in pregnancies that are characterized by high levels of estrogen and progesterone (126). Treatment of female rats with estrogen and progesterone can result in increased endotoxin levels and can also increase the level of TNF-α and CD14 in Kupffer cell (127) with increased gut permeability (128). Another study has indicated that the estrogen serves as a good prognostic marker in case of gut injury whereas testosterone serves as a marker with bad prognosis (129). Thus, hormonal changes in the acute gut trauma models may be entirely different than in the chronic alcohol models. However, it is established that the role of estradiol treatment to potentiate alcohol-induced apoptosis can lead to increased gut permeability in vivo (130).

8. MICROBIOTA: GENDER SPECIFIC GUT-LIVER AXIS

The human body holds more than trillions of microorganisms. In the adult intestine, a total number of about 10^{14} bacterial cells are present which is ten times higher than the number of human cells in the body (131). The microbiota redeems a nutrient-rich environment in the mammalian intestine with mutual association. However, this dynamic habitat undergoes constant and rapid changes with lifestyle, hygiene, use of antibiotics, and alcohol consumptions that affect the gut microbial composition. The gut microbiota differs in men and women at the bacterial phyla level (Firmicutes/Bacteroidetes ratio), at the genus level (Bacteroides, Bilophila, Veillonella, and Methanobrevibacter), and at the species level (B. plebeius, B. cacaee, C. catus). The Firmicutes/Bacteroidetes ratio has a great importance in the development of obesity between genders (132). Apart from few controversial results (133, 134), most of the studies in human and in mice (135) have reported that this ratio is increased in obesity and in case of fatty liver (136–138). The abundance for Bacteroides-Prevotella group (together Bacteroidetes phylum) is higher in men (139). However, a recent study using Next Generation Sequencing (NGS) has shown that the women are characterized by a lower abundance of Bacteroidetes (140). Therefore, the conflicting results in term of intestinal bacterial proportions might be explained by the men/women ratio, range of age, and the grade of obesity in the different cohorts studied. In this context of obesity, a higher proportion of Firmicutes is found in women regardless of the Body Mass Index (BMI). Interestingly, a higher proportion of Firmicutes is found in men under a BMI of 33 whereas a lower proportion is detected when BMI is > 33, reflecting a potential sexual dimorphism in gut microbiota composition is also influenced by BMI (141). Moreover, the gender differences in the energy metabolism of liver and the endotoxin level are related to the differences in sex hormone levels (141) as well as a persistent effect on gut microbiota over time. In this context, the current interest is mainly focused on how the gut affects liver injury through the portal vein axis (142, 143).

Chronic liver diseases and HCCs are accompanied by the translocation of intestinal bacteria and PAMPs. Thus, it is apparent that the gut-liver axis is important for the development of liver injury. In case of acute alcoholic hepatitis, the serious complication of alcohol consumption allows the presentation of PAMPs to the hepatic macrophages (Kupffer cells) by modulating the intestinal permeability (Figure 2) (118). In the animal model of ALD, increased expression of TLR1, 2, 4, 6, 7, 8, and 9 is reported with increased sensitivity to their respective ligands (144) where as in humans, TLR2, 4 and 9 are upregulated in the neutrophils of the alcohol induced hepatitis patients (145), suggesting that the TLR signaling pathway is significant in the pathogenesis of the disease. In a murine model of ALD, the intestinal bacterial overgrowth occurs with a corresponding reduction in probiotic species such as Lactobacillus (146). In humans, bacterial overgrowth is found in the jejunal aspirates from the chronic alcohol addicts (147). Using the next generation sequencing of 16S ribosomal RNA (rRNA) (148) it is found that the patients with gut dysbiosis have shown significantly lower levels of Bacteroides and significantly higher levels of Proteobacteria which is associated with increased systemic endotoxin. Usually, the sterilised gut protects against alcohol-induced intestinal barrier leakage (149) but the disruption of tight junctions is probably mediated by the microbial metabolism of alcohol to acetaldehyde (150). The systemic TNFα and IL-1b, which are increased in the patients with alcoholic hepatitis, also reduce tight junction integrity so that a positive feedback loop may take place during alcohol abuse (151). In a more acute murine model of alcoholic steatohepatitis (ASH), Lactobacillus treatment has shown to restore the intestinal integrity, reduce oxidative stress and improve histological liver damage (152). Moreover, Chronic alcohol addicts have higher levels of endotoxin lipopolysaccharide.
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The composition of the gut microbiota may regulate the mechanism of excess energy storage in the body and this effect might be gender dependent. Moreover, the sexual dimorphism in total body fat content observed in rodents has shown to disappear in the germ free animals suggesting a role of the gut microbiota (156). Thus, it is reasonable that the gender-related differences regarding bacterial composition may have an impact on how the men and the women differentially store the excess energy as well as its reflection in the progression of aberrant diseases. It is unexpected that when we study the literature for probiotic treatment we find so few published studies that mention gender. One stimulating investigation establish that probiotic treatment was not actual in accelerating the clearance of Salmonella infection or in reducing acute symptoms. In women, L. plantarum treatment were related with greater abdominal pain, whereas in men it increased the presence of diarrhea symptoms in the post infectious stage. But regardless of treatment, women tended to clear Salmonella more rapidly than men, and after clearance women experienced loose stools, nausea, and flatulence more frequently than did men (157).

9. NON-ALCOHOLIC FATTY LIVER DISEASE

Fatty liver is the result of the deposition of triglycerides due to the accumulation of fatty acids in the hepatocytes. Lipid peroxidation products are generated due to the impaired β-oxidation of the accumulated fatty acids within hepatocyte mitochondria. The impaired fatty acid β-oxidation causes a reduced mitochondrial electron transport and activation of CYP2E1 in the microsomes. The elevated CYP2E1 and the defects in mitochondria induce the ROS formation and lipid peroxidation which in turn initiate hepatic cell death via ROS-induced DNA injury. The membrane lipid peroxidation and discharged products of lipid peroxidation namely malondialdehyde (MDA) and 4-hydroxynoneal (HNE) accumulate into the space of Disse of liver. Both the MDA and HNE are able to activate inflammatory cells (neutrophils, macrophages and Kupffer cells) and HSCs. Activated inflammatory cells in turn produce chemokines, TNF-α, and ROS. Several chemokines like interleukin-8 (IL-8) and monocytes chemo-attractant protein-1 (MCP-1) attract lymphocytes, monocytes, macrophages, neutrophils, and Kupffer cells to the inflammatory sites leading to the persistent liver injury. In an environment of chronic inflammation in fatty liver diseases, the heterogeneous population of different subsets of liver dendritic cells (DCs) are transformed to potent inducers of immune responses (158). The coordination of lipid metabolism and DCs function suggests that immunogenic DCs are associated with liver lipid storage, representing a possible pathophysiological mechanism in the development of Non-alcoholic fatty liver disease.

NAFLD is a group of diseases defined by a hepatic fat infiltration in more than 5% of hepatocytes in the absence of excessive alcohol intake. It includes a histological spectrum ranging from simple steatosis to non-alcoholic steatohepatitis (NASH). NASH is classified by the hepatocellular damage due to steatosis and lobular inflammation (159) without having significant alcohol consumption as well as without viral, autoimmune, and congenital liver disease markers. However, corresponding increase in the occurrence rates of other metabolic syndrome like hyperglycemia, visceral obesity, hyperlipidemia, and hypertension is observed in NASH (160). Abdominal fat tissue is a major source of free fatty acids and cytokines in the liver. Accumulation of fat favors the early development of insulin resistance, dyslipidemia, and high blood pressure. No differences are observed in the accumulation of hepatic and intra-abdominal fat between men and women, but this is affected by dietary lipid consumption (161). The Visceral adipose tissue accumulation is found more rapidly with age and weight gain in males and postmenopausal females than in younger females (162). Female needs a higher degree of adiposity to achieve the same metabolic disturbances as men probably due to the attribution of their sex hormones (163). In the postmenopausal women, the distribution of the body fat changes toward visceral adiposity (164). However, few studies have reported that the NAFLD is approximately 1.5 times more prevalent in females than in males (164, 165) and the female gender is at higher risk for NASH (166). Moreover, current studies have demonstrated that the endogenous estrogen has a protective role against NASH which may explain why the prevalence of NAFLD increases in women over 50 years of age (167). However, the probable role of estrogen in the hepatic lipid metabolism and fibrosis require further investigation.

9. OBESITY AND METABOLIC LIVER DISEASES

9.1. Gender inconsistency in obesity

Currently, the medical consequences of the obesity contribute a major burden of healthcare costs. All the obese individuals are at equal risk of obesity-induced cardiovascular and metabolic diseases. Obese women of reproductive age are relatively protected from cardiovascular diseases as compared to the age-matched men (168). Gender differences in the proliferation and differentiation of the pre-adipocytes, the adipose tissue development, and the modulatory effects of sex steroids on the adipocytes regulate the obesity associated metabolic
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diseases (169). Moreover, a strong connections do exist between the chronic inflammation induced by obesity and the diseases such as atherosclerosis, type 2 diabetes, cancer, and autoimmune diseases (170). Activation of myeloid cells such as monocytes, macrophages, and neutrophils are noticeable characteristics of the obesity in both human and animal models (171). The enhanced myelopoiesis with high fat diet in male mice is a substantial mechanism to generate obesity-induced inflammation (172–174). Furthermore, the obesity influences the expansion of the hematopoietic stem cells (HSCs) and myeloid progenitors with a consequent increase in activated monocytes (Ly6chi/CCR2) (175, 176). These monocytes traffic to obese adipose tissue and form CD11c positive M1-like adipose tissue macrophages (ATMs) that produce activated cytokines and an inflammatory environment contributing to the insulin resistance (177). Several preclinical studies in rodents have identified a blunted pattern of inflammatory gene expression in the female visceral adipose tissue with diet-induced obesity (DIO) (178–180) that correlates with a decrease in adipose tissue inflammation in the obese female mice compared with their male counterparts. Although females have a robust humoral immune system mediated response in many autoimmune diseases (181) as compared to males, they have shown to attenuate the innate immune responses to the systemic infections and a lower incidence of severe sepsis (182). In fact, the peripheral blood monocytes (PBMC) from female patients produce less TNF-α in response to LPS activation compared with males (183). Several studies have demonstrated the differences in male and female adipose tissue biology through the gene expression profiles and lipid processing, adipose tissue macrophages, and leukocyte development. Singer et al have compared and contrasted the effects of high fat diet-induced obesity on glucose metabolism and leukocyte activation in multiple depots in male and female mice (184). Interestingly, the male mice have demonstrated an increased weight gain and CD11c positive ATM content compared with female mice after giving both short term and long-term high fat diet (HFD) in spite of similar degrees of adipocyte hypertrophy. They have also performed competitive bone marrow transplantation (BMT) experiment to compare myeloid cell activation between sexes in the same recipient and have found that both male and female bone marrow from HFD-fed animals produce an increased quantity of myeloid colonies but fewer colony forming units (CFU) are generated in HFD females compared with males. After stimulation with LPS, the male bone marrow derived macrophages and dendritic cells produce more TNF-α and IL6 as compared to females (184) suggesting an immunological vulnerability on male in terms of obesity.

9.2. Metabolic syndrome

Metabolic syndrome (MS) is also a risk factor for cardiovascular diseases that affects public health in recent years (185). There are several data in the literature regarding the gender specific MS. It is mostly accepted that a higher incidence of MS is observed in men than in women with few exceptions (186). The occurrence of MS increases with the age of the general population and is more likely to occur in black and Hispanic female populations.

MS is closely connected with two types of white adipose tissues named as visceral adipose tissue (VAT) and subcutaneous adipose tissue (SCAT). Gender differences occur in the distribution and composition of body fat which help to explain the cause for the markedly higher HCC incidence in men. Men acquire nearly 30% more visceral fat than women (187, 188). Adipocytes are responsible for the storage of excess energy intake and the absorption of free fatty acids (FFAs) and triglycerides. Excess absorption of fat enlarges adipocytes and stimulates the release of FFAs (189). Linoleic acid, a particular form of FFAs, usually accumulates in NAFLD and has recently been known for the disruption of mitochondrial function, oxidative damage, and the selective loss of intra hepatic CD4+ T cells (190). On the other hand, the adiponectin is considered as a protective cytokine for cancer progression involving anti-inflammation, sensitization of insulin signaling, and anti-angiogenesis. Adiponectin induces the production of IL-10, inhibits the inflammatory factors like IL-6 Akt/STAT3 signal transduction and the NF-κB pathway cascade to promote apoptosis in HCC cells (189, 191–193). Adiponectin is expressed at a higher level in women than in men (194, 195) Excess visceral adiposity is complicated with various negative effects including insulin resistance and the elevated production of pro-inflammatory molecules such as leptin (LEP), resistin, tumor necrosis factor-α (TNF-α), IL-6, hypoxia-inducible factor1 (HIF-1), and adipocyte fatty-acid binding protein (A-FABP) (194–197) which is more common among male. Enlarged adipocytes also liberates FFAs to stimulate adipose tissue macrophages (ATM) and myeloid-derived suppressor cells (MDSCs) to produce TNF-α which in turn activate the adipocytes to undergo lipolysis and secret multiple cancer-causing adipokines (198). Estrogen and estrogen receptor (ER) signaling have been found to have a protective role in inhibiting the lipolysis of adipocytes (199). Estrogen substitute therapy helps postmenopausal women to get more adiponectin in the circulation (200). In contrast, androgen is shown to have a suppressive effect in adiponectin level consistently in cell lines, animal models, and human epidemiological studies (195, 201) suggesting the negative impact of androgen in the liver diseases (Figure 3). Altogether, this may explain the higher
possibility of chronic inflammation and the insulin resistance in men.

10. IRON METABOLISM AND GENETIC HEMOCROMATOSIS

Iron is essential for life because it is a vital component of the heme in hemoglobin of red blood cells, myoglobin, and cytochromes. It also serves as an essential cofactor for non-heme enzymes such as ribonucleotide reductase, the limiting enzyme for DNA synthesis (202). The average daily dietary iron intake of an adult person should be approximately 11–15 mg. Under physiological conditions, iron is consumed by the immature red blood cells in the bone marrow for heme biosynthesis and the rest is distributed in other tissues (202). However, the excessive iron is toxic because of its influence to produce ROS that can react readily with lipids and DNA leading to DNA mutagenesis and cell death. There is no regulated pathway for the excretion of iron in the body except by blood loss or desquamated intestinal cells. The amount of iron loss is nearly 1 mg per day. Iron deficiency is predominant in females within the reproductive age mainly due to the physiological loss of blood by menstruation and pregnancy. Based on the differences between the amount of iron available for the absorption and the increased requirement for iron, most females before menopause do not have adequate iron stores (65, 203). The liver stores iron and it plays a significant role in iron homeostasis in the body, while liver is particularly susceptible to excess iron levels. Liver also synthesizes hepcidin, the iron regulatory hormone (204), to plays a central role in the regulation of iron metabolism. An increase in hepcidin levels can lead to a decrease in duodenal iron absorption and the mobilization of reticuloendothelial iron stores, accomplished with hepcidin binding to the iron exporter, ferroportin, and inducing its internalization and degradation. Still it is not clear whether the female and male differ in the level of human hepcidin expression in the liver. However, the female mice express higher levels of hepcidin in the liver than males (205). Oxidative stress induced by

Figure 3. Gender dimorphism influences alter the metabolic pathways with visceral adiposity during liver injury. Visceral adiposity (VAT) can be observed more commonly in men and raise a pro-inflammatory microenvironment in the liver. VAT increases the release of pro-inflammatory adipokines, IL-6, TNF-α (tumor necrosis factor-α), HIF-1 hypoxia-inducible factor-1), Leptin, A-FABP (adipocyte fatty-acid binding protein) and reduces the protective counterpart like adiponectin, causing selective loss of intra hepatic CD4+ T cells, other immune cells (B lymphocytes, macrophages etc) infiltration, liver injury, and the subsequent pathological syndromes. The increase in the release of FFAs and gecolr to the liver from visceral fat also promotes insulin resistance, lipid peroxidation. The release of a pro-inflammatory cytokine IL-6 from hepatic stellate cells is also associated with an increased level of FFAs in VAT. Estrogen (primary female sex hormone) plays a protective role inhibiting VAT to reduce chronic inflammation in metabolic syndromes. But the suppressive role of androgen (male hormone) on adiponectin may cause more chronic inflammation and increase the probability of HCC (195). MDSCs: myeloid-derived suppressor cells.
HCV proteins and alcohol is suggested to be one of the mechanisms responsible for the down-regulation of hepcidin expression (206). In addition, inflammation is a foremost and vigorous inducer of hepcidin gene transcription regardless of body iron levels (202). The more pronounced down-regulation of hepcidin among males is abrogated by the antioxidants. In contrast, estradiol inhibits the spontaneous secretion of proinflammatory cytokines in murine peritoneal macrophages in female (205) determining the fact that besides iron, hepcidin may be also regulated by the female gender-specific factors including estrogen (Figure 4).

The genetic hemochromatosis (GH) is a prevalent iron overload disorder where patients absorb more than the normal amount of iron through the intestine. It is demonstrated that the men accumulate more iron and have a higher incidence of liver injury due to extrahepatic deposition of iron (207, 208). Because of this reason, serum ferritin levels are higher in men. Consequently, the clinical appearance of GH is different between men and women.

11. AUTOIMMUNE DISEASES: GENDER SPECIFICITY

Based on a constellation of clinical, serologic, and liver pathology study, the three major well-defined categories of autoimmune liver disease are autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC), and primary sclerosis cholangitis (PSC). Although these diseases are considered autoimmune in nature, the etiology and possible genetic and environmental triggers of each remain incomprehensible. Numerous studies have determined the gender differences in the immune system and suggested that the estrogen and androgen may modulate the immune system (Figure 5). Females have a significantly higher number of CD4+ T lymphocytes and a higher CD4+/CD8+ ratio than men (209). The secretion of interferon-γ (IFN-γ) and interleukin (IL)-10 from T cells is also tend to
increase after addition of estrogen (210). In contrast, the androgen inhibits the secretion of IFN-γ, IL-4, and IL-5 from T cells in the murine model (211).

Autoimmune hepatitis (AIH) is a stage of liver disease characterized by the progressive inflammatory destruction of the parenchyma in association with circulating autoantibodies and hypergammaglobulinemia. Like most autoimmune disorders, AIH is also common in female patients with a male: female ratio of roughly 1:4. It can present at any age, but younger female appears to have a more severe form of the disease. Both genetic and environmental factors are involved in autoimmune hepatitis, but the failure of impaired regulatory T cells to control immune reactions against liver host antigens is approximately four times higher in the female than the male (6). It is found that after six month of corticosteroid treatment in men with AIH, there is a minor frequency of normalization of ALT stages (212) which, in contrast, appears to have a better long-term survival and outcome than women (213). In contrast, the severity of AIH in females is found to be less during the second trimester of pregnancy when the estrogen is secreted at a high levels and with high probability of acute AIH exacerbation after delivery (214) due to inflammatory milieu (104).

Primary biliary cirrhosis (PBC) is a chronic cholestatic liver disease characterized by fibrosis
along with immune-mediated inflammatory demolition of the small intrahepatic bile ducts. It can progress to cirrhosis and subsequent liver failure (215–217). PBC is a distinctive female disease that usually occurs after age of 40 with an average incidence rate in women of 10:1 (218). This incidence rates in women and men range from 3:1 to 22:1 (218). Quite a few studies have recognized an increased incidence of X haploinsufficiency in female patients (219, 220). But, the effects of sex hormones on the synthesis of antibodies and cytokines during maturation and activation of lymphocyte have been suggested as significant contributing factors. In fact, the immunomodulatory effects of estrogen during reproductive life are known as a distinct factor (221, 222). One study have also established how Y chromosome loss is associated with the PBC in male patients (223). The current scenario also have suggests that the epigenetic changes may be perfect targets for new personalized treatments of PBC. However, still no convincing evidence has supported any of these predictions. Females experience more inflammatory responses often than males. Moreover, the female sex hormones are linked with pruritus. In addition, female sex hormones may cause more malaise, anorexia, weight loss, and fatigue. In contrast, males experience more jaundice, jaundice with pruritus, and upper gastrointestinal bleeding. Concomitant autoimmune diseases such as Sicca Syndrome, Scleroderma, and Raynaud’s phenomenon are shown to be less prevalent in men. Significant complication are also found among men in hepatocellular carcinoma (HCC) with PBC (224).

The progressive inflammation and fibrosis of the intrahepatic and extrahepatic bile ducts can also develop chronic cholestatic liver disease called Primary sclerosis cholangitis (PSC) that eventually leads to decompensated cirrhosis (225, 226). PSC is predominant among men but the pathophysiology of PSC is very uncertain because of its complex immune-mediated responses. It may occur due to persistent immune mediated damage to cholangiocytes and/ or progressive destruction of bile ducts with the environmental exposure.

12. HORMONAL CONTROL AND PRIMING OF HEMATOPOIETIC STEM CELLS

Commonly, diseases do not affect males and females equally, despite demographic sex ratios. Due to dramatic hormonal changes in the various stress responses within male and female, research studies become challenging other than findings of reproduction and fertilities. For this sex dimorphism, the profile of growth hormone secretion can alter significantly in normal condition (227). The growth hormone is continuously secreted in female rats and the hormone levels are always detectable in the circulation, while in male rats, it is secreted by episodic bursts every 3.5 to 4 hours with low or undetectable levels between the peaks. Integrated 24 hour growth hormone secretion and fasting blood growth hormone levels are higher in female than in male (228). Estrogen stimulates the growth hormone secretion (227). The high dose of transdermal estrogen administration in menopausal women increases integrated growth hormone secretion (229). Most interestingly, the growth hormone increases ADH activity in the liver. The stable exposure of growth hormone to the hepatocyte cultures results in an increased ADH activity that resembles the female pattern of growth hormone secretion. As compare with human studies, the ADH activity is also higher in female rats and mice than in their male counterparts (230). Thus, the increased rates of toxic acetaldehyde production in females compared with males may be responsible for the known increased susceptibility to alcohol-induced liver injury. In contrast, the female estrogen acts as a potent endogenous antioxidant (231) that suppresses the hepatic fibrosis and attenuates the induction of redox sensitive transcription factors, hepatocyte apoptosis, and stellate cells activation by inhibiting the generation of ROS and TGF-β in primary cultures (111, 232, 233). It is found that the hepatic steatosis spontaneously becomes evident in an aromatase-deficient mouse which lacks the intrinsic ability to produce estrogen and is impaired with respect to hepatocellular β-oxidation of fatty acid in the animal model. Estrogen replacement reduces hepatic steatosis and restores the impairment of mitochondrial and peroxisomal fatty acid β-oxidation to a normal level (234). The tamoxifen, the well-known antiestrogen, mostly used for estrogen receptor-positive breast cancer, has been shown to be associated with an increased risk of developing fatty liver and NASH in such patients. There is long term debate on how hormone influences the mobilization of the tissue specific stem cells and coordinates the long term signals in response to the body’s need or organ injury (235). During pregnancy, the increases in estrogen and progesterone levels coordinate an expansion of mammary stem cells which is required for remodeling of the mammary gland. The increases of hormone prolactin stimulate the production of pancreatic β-cells (236) and the proliferation of neural stem cells (237) that causes the increased metabolic load of the pregnant female. It has been shown in mice that stem cell derived from female have higher muscle regeneration efficiency than male. In another study of human hematopoietic stem cell transplantsations, superior survival is observed with maternally donated recipients as compared with recipients of paternal transplantsations (238).

Hematopoietic stem cells (HSCs) are originally isolated from the bone marrow in the early 1960s and have been used in the cell therapy to reconstitute blood cells and restore the immune system. Hematopoietic stem and progenitor cells
(HSPCs) are multipotent and self-renewing stem and progenitor cells which can form different types of blood and immune cells. HSPCs can give rise to the cells of the erythroid, lymphoid, and myeloid lineages during the hematopoiesis (239). However, there is no clear cut evidence on the proliferation and differentiation of HSPCs in sexually dimorphic function. Current interesting research findings have suggested that the estrogen positively acts on HSPCs proliferation and HSCs from female mice divide more frequently than those from the male mice. However, the basal number of the HSCs in the bone marrow and spleen have remained the same because the cell death of those newly generated HSCs in female is also higher as compared to males (240). The percentage of proliferating HSCs has shown to increase further during the pregnancy (Figure 6) (241). The proliferation of HSCs can be endorsed in males and ovariectomized females by injecting estradiol. It is also demonstrated that the Esr1, the gene coding oestrogen receptor-α (ERα), is highly expressed in HSCs and is essential for the increased proliferation of female HSCs during pregnancy (241). It could also be concluded from the previous studies that the enhanced engraftment (242) of human HSCs transplants in female as compared to the male mice is due to estrogen (243). In addition, the telomere shortening observed in human bone-marrow transplants from female donors might reflect a greater requirement of female HSCs to guard against the exhaustion (244). This is entirely new concept that the long range signals which act not only in response to specific systemic needs, but also under basal conditions to keep hematopoietic stem cells in a primed state, ready to act when pregnancy is initiated with hormonal fluctuations.

13. LIVER TRANSPLANTATION

13.1. Impact of Gender on Donor and Recipient

A number of studies have reported the role of gender in the setting of liver transplantation (LT). It has been shown that in comparison with men, women have, on average, lower median serum creatinine levels, estimated glomerular filtration rates, and mean MELD (Model for End-Stage Liver Disease) scores. After adjustments for appropriate covariates, women are found to be less likely to undergo LT than men and they have a higher mortality rate (245). A comparative study also has demonstrated that the women are more prone to die than men or become very sick due to LT (246). The recipients of gender mismatched grafts have an 11% higher risk of graft loss (247). According to few studies, female-to-male mismatch is associated with a 17% increased risk of graft loss, but male-to-female mismatch is not significant (247, 248). So, the donor quality differs significantly between females and males. The donor risk index is significantly higher in female than in male. In the animal models, it has been observed that the female rats develop a greater...
degree of hepatic tissue lactic acidosis during warm ischemia in comparison with males (249). This work has suggested a potential metabolic explanation for the worse outcomes of recipients with female donors due to sustained liver injury from increased acidosis. Another study has shown that both female and male livers from rats given β-estradiol present with a significant accumulation of lactates and this explains that the mechanisms for gender difference in the liver’s metabolic responses to ischemia are estrogen and other male hormone mediated. (250) Previous studies have also confirmed that the women have better long term survival after LT than men, but older age (>65 years) is associated with worse outcomes (251).

13.2. Disease recurrence among male and female after Transplantation

Several reports on non-alcoholic and alcoholic viral hepatitis have suggested that the disease reappearance after LT can be influenced by the gender. Approximately 50% of patients after LT have shown NASH (252) whereas 5–10% cases progress to cirrhosis (253, 254). MS is also described in 48–50% of patients followed for more than 6 months after LT which is also associated with a higher risk of cardiovascular and cerebrovascular events. Interestingly, MS serves a significant risk factor of the mortality in postmenopausal women and premenopausal women (255). The incidence of diabetes has shown to increase in many cases after LT (256, 257) but this is also associated with older age and not with menopausal status (258). The obesity is commonly associated with LT (259) where the central adipose tissues are typically evident as an increased abdominal girth (260). Alcohol relapse after LT remains a challenge mainly because of the lack of analytical factors of the heavy drinking patterns (261, 262). A recent study have reported that the females tend to resume alcohol consumption more frequently than men (25.9.% versus 16.7.%), although the difference is not statistically significant (263). The recurrence of HCV after LT is universal within nearly two-thirds of patients (264). Female gender has been described as a risk factor for severe HCV recurrence after LT and graft loss. In general, females infected with HCV have a slower rate of fibrosis progression and a lower rate of cirrhosis than men (265). So, it can be interpreted that the women who need LT have significant virological, genetic, and immunological factors responsible for severe HCV disease which in turn causes the rapid recurrence of HCV after LT. Another interpretation is that due to postmenopausal immune-competency of most of the case of HCV the LT, women show more frequent steatosis, a typical factor for recurrence (266, 267). In fact, the female recipients have a higher risk of fibrosis progression than men after antiviral therapy (268).

13.3. Immunosuppression and Osteoporosis

Several concerns exist regarding the immunosuppression after LT. Immunosuppressive drugs cause bone loss (268, 270). Calcium is a vital component required for deposition of bone mineral throughout life. Our body stores more than 99% of its calcium in the bones and teeth. Imbalance calcium level with vitamin D in bone and the extracellular fluid (ECF) or plasma causes osteoporosis (271). High dose of steroids after LT tend to reduce bone formation due to decreasing osteoblast replication and differentiation (272, 273). Cyclosporine, the calcineurin inhibitors, has adverse effects that can increase bone turnover (274). It is also found that the tacrolimus may cause less bone loss in humans than cyclosporine (275, 276). Due to decreased serum estrogen levels, the postmenopausal female after LT are at higher risk for developing osteoporosis than women with regular menses as well as male (277).

14. THERAPEUTIC IMPLICATION ON DISEASE MANIFESTATION

14.1. Gender specific disease management

The clear diversities of gender in occurrence, presentation, natural history, and outcomes exist for common liver diseases and HCC. These disparities are significant for the clinician to identify as they influence the likelihood of a given diagnosis for a patient and the potential for progression of the liver disease. Gender medicine focuses the scientific community towards the understanding and analyzing clinical, patho-physiological, prevention, and the treatment differences in diseases that are equally represented in men and women (278). The determinations of these fields are to deliver the best treatment to the individuals based on the scientific evidence. Gender specific studies are importance not only to measure the drug or alcohol doses but also to administer an appropriate management of viral chronic hepatitis during pregnancy as well as to summarize the strategies to prevent mother-to-child transmission. It is also important to focus on maternal and perinatal outcomes, disease progression, and its impact on pregnancy. Focuses should also be given to effectively design new effective drugs to prevent maternal infection transmission without significant adverse effects or complications. The protective role estrogen has been demonstrated here with the evidence that the menopause is associated with an accelerated rate of fibrotic progression providing the idea of hormone replacement therapy to minimize this effect (279). The diverse observation of gut microbiota between men and women is relevant for the proper understanding of the basis of gender differences in the prevalence of metabolic and intestinal inflammatory diseases. However, additional studies are needed to unveil...
the specific mechanisms such as sex steroid milieu, gonadal status, or genetic factors etc.

14. 2. Stem cells: A new era of cell therapies

Cell therapy is a developing form of treatment for several liver diseases but is limited by the availability of donor livers. Cell-based therapeutics may include cell transplantation, gene therapy, bioartificial liver devices, or bioengineered organs. Different cell lines have been used experimentally to support liver function and treat inherited metabolic disorders, acute liver failure, cirrhosis, liver cancer, and small-for-size liver transplantsations. So, the most promising approach on Stem cells would be to provide an alternative to the use of primary hepatocytes. The capacity of stem cells for differentiation and self-renewal make them a plausible source for the generation of unlimited numbers of hepatocytes. Consequently, the stem cell therapies as an alternative for whole-organ liver transplantation hold great promise for the treatment of liver disease. Numerous types of stem cells are known to be appropriate for liver cell replacement based on the characteristics of the different liver diseases. The stem cell therapy is thought to be ultimate treatment for degenerative diseases and liver cancer. Bone marrow derived stem cells comprise mainly hematopoietic and mesenchymal stem cells (MSCs). MSCs are multipotent progenitor cells found in bone marrow and other adult organs and tissues, such as adipose tissue, that are easily accessible and can be expanded rapidly in culture (280). MSCs are also known to have a higher potential for liver regeneration (281, 282). In addition, they offer another benefit over hematopoietic stem cells. For example, they have immunomodulatory or immunosuppressive properties that down regulate T cell, B cell, and NK cell function (282). The most elementary question in this context is whether the stem cells can reveal the sexually dimorphic function. Notta et al have demonstrated sticking results regarding the engraftment efficiency of hematopoietic stem cells. They have found that the engraftment of human hematopoietic stem cells is more efficient in female recipient (243). This can solve the long-term observation on the fewer women than men on the liver transplant waitlist in future. Stem cell functions can also be modulated by different systemic signals originating from the exercise, diet, circadian rhythm, mating, and pregnancy (283). However, the stem and progenitor cells are believed to have self-renewal function throughout the life and contribute to the repair and maintenance of the tissue.

15. CONCLUSIONS

In general, men are more likely to die from chronic liver disease, cirrhosis and hepatocellular carcinoma than women. But women appear to be more vulnerable than men in many adverse consequences of acute liver injury such as alcohol exposure, drug overdose as well as the autoimmune diseases. Even liver transplant occurs less commonly in women than in men. Thus, sexual dimorphism in the mortality, morbidity, disease progression, pathology, phenotype expression, and molecular signaling can be the matter of plentiful research. However, the drug treatment schedule and dosages do not distinguish between men and women despite evidence of pharmacokinetic and pharmacodynamic difference between the sexes (10, 284, 285). These findings indicate a necessity for a special attention to sex differences, which is essential for the understanding of the basic cellular and stem cell biology, biology of several diseases as well as the regenerative medicine. This could be one of the necessary steps towards the path of personalized healthcare. Finally, the well broadcasted research into gender differences will benefit everyone, both the women and the men.

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Abbreviations: ACLF, Acute and chronic liver failure; ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; AH, alcoholic hepatitis; AIH, Autoimmune hepatitis; ALD, alcoholic liver disease; ALF, Acute liver failure; ALT, alanine aminotransferase; ASH, alcoholic steatohepatitis; α-SMA, α-smooth muscle actin; ATM, adipose tissue macrophages; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B viral protein; BMT, bone marrow transplantation; CHB, chronic hepatitis B; CHC, Chronic hepatitis C; CYP2E1, cytochrome P450 2E1; DAMPs, damage associated molecular patterns; EGFR, epidermal growth factor receptor; ER, estrogen receptor; GH, genetic hemochromatosis; HBV, hepatitis B virus; HCV, C hepatitis C virus; HCC, hepatocellular carcinoma; HSCs, Hepatic stellate cells; HSPCs, Hematopoietic stem and progenitor cells; IL-1, Inflammatory Interleukin 1; IFN-γ interferon-γ; LT, liver transplantation; MyD88, myeloid differentiation primary response gene 88; MS, Metabolic syndrome; NAFLD, Non-alcoholic fatty liver diseases; NASH, Non-alcoholic steatohepatitis; NF-κB, Nuclear factor kappa B; PAMPs, pathogen associated molecular pattern; PBC, Primary biliary cirrhosis; PSC, Primary sclerosis cholangitis; ROS, Reactive oxygen species; TGF-α, Transforming Growth Factor alpha; TLR4, Toll-like receptor; TNF-α, Tumor necrotic factor-α

Key Words: Fibrosis, Cirrhosis, HCC, Acute liver disease, Chronic liver diseases, Kupffer cells, Hepatic stellate cells, Review

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