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Smoking as a risk factor for autoimmune liver disease: what we can learn from primary biliary cirrhosis

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ABSTRACT

Primary biliary cirrhosis (PBC) is a cholestatic liver disease characterised by the immune-mediated destruction of biliary epithelial cells in small intrahepatic bile ducts. The disease is characterised by circulating anti-mitochondrial antibodies (AMA) as well as disease specific anti-nuclear antibodies (ANA), cholestatic liver biochemistry, and characteristic histology. The disease primarily affects middle-aged females, and its incidence is apparently increasing worldwide. Epidemiological studies have indicated several risk factors for the development of PBC, with family history of PBC, recurrent urinary tract infection, and smoking being the most widely cited. Smoking has been implicated as a risk factor in several autoimmune diseases, including the liver, by complex mechanisms involving the endocrine and immunological systems to name a few. Studies of smoking in liver disease have also shown that smoking may progress the disease towards fibrosis and subsequent cirrhosis. This review will examine the literature surrounding smoking as a risk factor for PBC, as well as a potential factor in the progression of fibrosis in PBC patients.

Key words. Autoimmunity. Autoimmune disease. Cigarette. Liver. Prevention. Smoking.

INTRODUCTION

Primary biliary cirrhosis (PBC) is characterised by the autoimmune destruction of small intrahepatic bile ducts, with fibrosis progressing to cirrhosis and eventual liver failure.[1-6] Patient presentation varies, with some patients being asymptomatic with liver function tests indicating cholestasis.[2,7-10] Symptomatic patients typically present with pruritus, fatigue and arthralgias,[2,10,11] with more severe symptoms being related to portal hypertension, including ascites, jaundice, or variceal bleeding.[2,10,11] The diagnosis of PBC is based on biochemical markers of cholestasis, histological features of PBC, and the presence of disease specific anti-mitochondrial antibodies (AMA) and/or disease-specific anti-nuclear antibodies (ANA).[1-3,7-10,12-31] AMA is pathognomonic for PBC with the presence of the disease is questioned in their absence, and it is commonly the case that AMA is predictive of future disease development in asymptomatic individuals.[1-3,7-10,12-22,26,28,32] These autoantibodies also show a higher prevalence among family members of patients with PBC, and AMA-positive individuals are at risk of developing PBC.[33] AMA specific to PBC are directed against components of the 2-oxo-acid dehydrogenase complexes (previously known as M2 antigens), primarily the E2 subunit of the pyruvate dehydrogenase complex, or PDC-E2.[2,8,9,13,25,34-38] Medical treatment of PBC includes urseodeoxycholic acid, which frequently leads to a good biochemical response and decreased indices of cholestasis in those diagnosed in the early stages of the disease, or liver transplantation in more severe cases.[1-3,5,10,39-46]

The aetiology of PBC is not fully understood, but it appears that a variety of factors including genetics, immunological factors, as well as exposure to xenobiotic and infectious agents may ac-

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count for the development of the disease.[17,47-61] Genetic and genome wide association studies (GWAS) have demonstrated associations with several HLA and non-HLA regions.[54] Associated regions include HLA-DRB1, DQA1, DQB1, and DQA2, and non-HLA regions such as IRF5, SPIB, the IKZF3-ORMDL3 of chromosome 17q12-21, IL12A and IL12RB.[54,62] IRF5-TNPO3, 17q12-21 and MMEL1 have been found to be associated with primary biliary cirrhosis in other studies.[50] The most recent GWAS on PBC included 1,840 PBC patients and 5,163 controls from the UK, identified twelve new susceptibility loci including new candidate genes STAT4, DENND1B, CD80, IL7R, CXCR5, TNFRSF1A, CLEC16A and NFKB1.[58]

Infectious agents have been implicated, largely through mechanisms such as molecular mimicry and cross reactivity.[4,7,9,12,36,63-79] Other compounds including chemicals have also been implicated in the development of PBC, including contamination of air, water, and soil.[80,81] Several studies have consistently indicated that several risk factors for PBC development, including recurrent urinary tract infection and smoking.[82-87] Smoking has been implicated in several autoimmune diseases, and its effects may be multifactorial, involving tissue damage and apoptosis, inflammation, and anti-oestrogenic effects.[88-101] This review will examine the role of smoking as a risk factor for the development of PBC, as well as the implication of smoking in regards to liver fibrosis (Table 1).

EPIDEMIOLOGICAL STUDIES

Over the past decade, epidemiological studies on PBC have identified multiple risk factors for PBC development, ranging from familial and past surgical history, to nail polish use and oestrogen deficiency.[48,82,84-86] Several risk factors, however, have been indicated in virtually all major epidemiological studies, namely urinary tract infection, family history and smoking.[82,84-86] Howel, *et al.* conducted a population-based case-control study of PBC in the North East of England, using postal questionnaires covering medical history and lifestyle sent to 100 PBC patients and 223 controls.[102] An unexpected association was found with smoking, where 76% of PBC and 57% of controls had ever smoked.[102] Parikh-Patel, *et al.*[85] conducted a study on 199 PBC patients, 171 of their siblings, and 141 of their friends as controls. They reported that 66.2% of PBC patients had a history of smoking, compared to 62.2% of their siblings and 49.9% of their friends.[85] Prince, *et al.* also found an association between PBC and smoking in their study, comprised of two PBC groups: one from an epidemiological study consisting of 318 cases, and the other from a PBC support group consisting of 2,258 cases.[86] The odds ratio (OR) for those who had ever smoked was 1.63 in the epidemiological group, and 1.57 in the support group.[86] Multivariate analysis demonstrated an adjusted OR of 1.6 compared to the epidemiological cases, and 1.5 compared to the support group cases.[86]

Table 1. Evidence in support and against the role of cigarette smoke in the pathogenesis of primary biliary cirrhosis (PBC).

Evidence in support of smoking in the pathogenesis of PBC	Evidence against smoking in the pathogenesis of PBC
Associations found in all large epidemiological studies	Lack of apparent mechanism for the induction of biliary pathology
Advanced fibrosis associated with a significant smoking history	Variability between epidemiological reports
Increased risk of advanced fibrosis with increased number of pack years	Liver enzyme levels did not differ between smokers and non-smokers
Significant hepatic inflammatory activity seen in smokers vs. non-smokers	Interlobular bile duct paucity more severe in non-smokers
Increased IFN-γ and IL-10 in smokers, reflecting an increased Th1 response	No difference in immunoglobulin or autoantibody levels between smokers and non-smokers
Chemicals in cigarette smoke also implicated in PBC clusters	

One of the largest, most comprehensive epidemiological studies on PBC was conducted by Gershwin, *et al.*,[84] involving 1,032 PBC patients from 23 different tertiary care facilities, and 1,041 controls. Controls were selected from random digit dialling, and both controls and PBC patients were administered a telephone questionnaire covering lifestyle and medical history.[84] Past smoking was found to be significantly associated with the development of PBC, with 60% of patients and 54% of controls having smoked more than 100 cigarettes in their lifetime.[84] Strangely, this association was related only to past smoking, as only 16% of PBC patients but 32% of controls currently smoking at the time the study was conducted.[84] This may be due to the cessation of smoking by PBC patients at the time of diagnosis, as Parikh-Patel, *et al.* found that 21.4% of patients stopped smoking at the time they were diagnosed with PBC.[85] Former smokers comprised 46.5% of the PBC group, compared to 36.6% of siblings and 34.8% of friends.[85] A higher percentage of individuals who had never smoked was found in controls groups, with 51.1% of friends and 37.8% of siblings having never smoked, compared to 33.8% of PBC patients.[85]

The above studies have indicated an association with past smoking and PBC. An epidemiological analysis conducted by Corpechot, *et al.*[82] went further and examined the role of active and passive smoking. That study involved 222 PBC patients and 509 controls, all of whom were administered a questionnaire which included details on exposure to cigarette smoke.[82] A history of cigarette smoke was defined as having consumed more than 100 cigarettes in a lifetime, and active smoking was defined as daily smoking for the past 6 months.[82] Active and/or passive smoking was found to be associated with PBC, with 45% of patients, and 19% of controls reporting either an active or passive smoking history.[82] Active smoking was reported in 20% of PBC patients and 13% of controls.[82] Persistent exposure to cigarette smoke was reported in 39% of PBC cases and 16% of controls.[82] Finally, a meta-analysis of five existing studies demonstrated that smoking, family history of PBC, and a personal history of urinary tract infection were strongly associated with PBC.[87] Those authors note that all studies included in the analysis were based on a Caucasian population, and that studies are needed in different populations to determine if there are varied risk factors depending on ethnic grouping.[87] As well, the cumulative effect of a number of potential risk factors is not clear, and has not been addressed in any of the above studies. For example, it is unknown as to how many patients with a history of urinary tract infection or family history of PBC, also had a significant smoking history. An analysis of these relationships may shed some light on the additive effects of a variety of triggers.

SMOKING AND FIBROSIS IN PBC

Epidemiological studies indicate that smoking, or at least a past history of smoking, is associated with PBC. Emerging evidence also appears to indicate that exposure to cigarette smoke may also have an impact on the progression of the disease towards fibrosis.[83,90,93,95,99,101,103-110] Previous studies in hepatitis C and alcoholic liver disease have indicated that smoking may impact the severity of fibrosis.[99,101,106,108,111] A study by Zein, *et al.*[109] attempted to determine the relationship between smoking and the severity of liver fibrosis at presentation in patients with PBC. Over a seven year period, 97 patients were retrospectively identified, and the cumulative number of cigarette packs smoked per year (pack-years) was calculated for each patient.[109] Advanced fibrosis (stage 3 or 4) was found to be associated with increased lifetime tobacco consumption, being greater than or equal to 10 pack years.[109] The association remained significant after adjusting for age, gender and alcohol intake, and cross-validation of 172 PBC patients confirmed these findings.[109]

A recent study has also demonstrated an association between smoking and advanced fibrosis, as well as biochemical and immunological changes.[83] A total of 223 PBC patients were interviewed regarding their smoking habits using a questionnaire, and histological data from presentation was available from 164 patients.[83] A significant smoking history was reported by 26% of patients (current or past history of smoking five or more cigarettes per day), 11% were active smokers, and 41% were non-smokers who had been exposed to cigarette smoke consistently, either at home or at work.[83] Advanced fibrosis (stage 3 or 4) was found in 37% of current smokers and 33% of past smokers, compared to only 16% of those who had never smoked.[83] In fact, each pack year of increase in smoking intensity was associated with a 5% increase in the likelihood of advanced fibrosis.[83] Florid destructive cholangitis was found to be more prevalent in past smokers (63%) followed by current smokers (61%) and non-smokers (57%).[83] Significant inflammatory activity was seen in 33% of smokers *vs.* 32% of non-smokers, and strangely, interlobular

bile duct paucity was less prevalent in current and past smokers (31 and 30% respectively) *vs.* non-smokers (54%).[83] Liver enzyme activities (ALP, GGT, AST, ALT) and concentrations of serum total bilirubin and albumin did not differ significantly between smoking and non-smoking groups.[83] No differences were observed in serum immunoglobulin levels, or in autoantibody titres.[83] Although further studies are needed to determine the mechanism underlying smoking as a factor for progressive fibrosis, it is recommended that patients with liver disease be encouraged to stop smoking.[83,103,112]

THE IMMUNOMODULATORY PROPERTIES OF SMOKING: A PATHWAY TO AUTOIMMUNITY?

The effects of smoking on the immune system are diverse, and the roles those changes play in autoimmunity have not been well defined.[113] Alterations in cytokine production, such as IL-13, and the subsequent balance of T lymphocyte subtypes have been implicated in epidemiological studies.[84-86] Gershwin, *et al.* note the possible effects of tobacco smoke on the Th1 cytokine response, which has found some support in regards to COPD, another autoimmune disease.[84,98,100] Whetzel, *et al.*[100] measured Th1 and Th2 immune responses via IFN-γ an IL-10 in 20 healthy non-smokers and 19 smokers aged 19-41 years. Smokers had increased IFN-γ and IL-10, even after 24 h of smoking abstinence.[100] IFN-γ levels were higher in female smokers, but no age or sex difference was noted with IL-10.[100] That study and others have demonstrated a predominance of the Th1 cell line in COPD,[100,114-117] and that the predominance of these cells may occur due to cigarette smoking. This is of interest in PBC, given that Th1 cells are the more prominent T-cell type of lymphoid infiltrates seen in PBC.[118]

Chemical compounds found in cigarette smoke have also been implicated in clusters of PBC. Ala, *et al.*[119] report a cluster of PBC patients near a superfund toxic waste site contaminated by volatile aromatic hydrocarbons. As there was no apparent contamination of groundwater, it was suggested that air contamination by chlorinated hydrocarbons, such as benzene, may be a potential factor in the development of PBC.[119] It is also possible that benzene-containing cigarette smoke may also be associated with PBC.[82,84,85,119] Immunological changes in response to exposure to chemical constituents of cigarette smoke, warrant further investigation on the experimental level.

CONCLUSION

The deleterious effects of smoking are now well known, and appear to be involved in the pathogenesis and progression of many diseases, including autoimmune diseases. The mechanisms involved are complex, and involve alterations in immunological and endocrine function, as well as the production of free radicals and oxidative stress. Epidemiological studies have strongly implicated smoking as a risk factor for PBC, and recent studies also indicate that smoking may also increase the risk of fibrosis in PBC patients. Although further studies are needed to confirm and clarify those studies, it is clear that smoking should be strongly discouraged among patients with PBC and other chronic liver diseases. Although it is not possible to 'un-do' the damage that smoking may have caused in patients at presentation, it may not be too late to educate patients on the possible effects of smoking on their cohabitating relatives. A family history of PBC has been shown to increase the risk of PBC, and therefore, action must be taken to reduce the risk of relatives developing PBC, such as by screening, but also in regards to harm reduction, such as reduction of smoking in the household. Although this may not prevent another diagnosis of PBC, it may have implications as to the severity of the disease at presentation, and hence patient outcome.

ABBREVIATIONS

- **AMA:** Anti-mitochondrial antibodies.
- **ANA:** anti-nuclear antibodies.
- **PBC:** primary biliary cirrhosis.
- **PDC:** pyruvate dehydrogenase complex.

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