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REVIEW ARTICLE

Hair dyes as a risk for autoimmunity: from systemic lupus erythematosus to primary biliary cirrhosis

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Abstract Environmental and genetic factors appear to be involved in the pathogenesis of primary biliary cirrhosis (PBC), a chronic cholestatic liver disease characterized by immune-mediated destruction of the small and medium sized intrahepatic bile ducts. Environmental factors include exposure to various infectious, xenobiotic and chemical compounds. These exposures may occur occupationally, through water or air contamination, pharmacological administration or by elective exposure, to name a few. Hair dyes are compounds that have been implicated in the development of several autoimmune diseases, including systemic lupus erythematosus (SLE) and PBC. So far, only epidemiological studies have addressed the role of hair dyes in PBC, with limited results. Hair dyes in SLE have been examined, and have recently demonstrated an association, both epidemiologically and immunologically. This follows a series of negative studies, which may not have taken into account several features of hair dye use. This review will examine the literature surrounding hair dye use and SLE, and compare this to data surrounding PBC. Treating physicians should be prepared for questions surrounding the need to take precautions against repeated hair dye use and this topic is discussed further.

Keywords Autoantibody · Autoimmunity · Cosmetics · Autoimmune disease · Environment · Genetics · Immunopathogenesis · Risk factors · Susceptibility

Abbreviations

AMA	Antimitochondrial antibody
ANA	Antinuclear antibody
PBC	Primary biliary cirrhosis
PPD	*P*-phenylenediamine
SLE	Systemic lupus erythematosus
UDCA	Urseodeoxycholic acid

Introduction

Environmental factors combined with genetic susceptibility are largely believed to be essential working partners in the development of autoimmune disease [1–4]. The term 'environmental factors' or 'environmental agents' in the discussion of the pathogenesis of autoimmune disease, is a blanket term to address environmental components such as microbial agents, vaccines, diet, drug exposure, heavy metals, ultraviolet radiation and smoking, among many others [3–9]. Monozygotic concordance rates below 50% are indicative that environment as well as genetics are involved [10–13]. As well, studies on genetically similar populations living under different conditions have demonstrated different incidence rates of autoimmune disease. The induction of autoimmune disease by these exposures may be through various mechanisms such as the alteration of autoantigen structure, altered expression of

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antigens, stimulatory effects on the immune system, T-cell dysregulation, apoptosis-mediated autoimmunity, molecular mimicry and immunological cross-reactivity [5, 14–18].

Cigarette smoking is one example of non-infectious agents which have been studied in rheumatoid arthritis (RA), autoimmune thyroiditis, inflammatory bowel disease, and primary biliary cirrhosis (PBC) [19–22]. Cigarette smoke has been shown to increase the production of pro-inflammatory cytokines, and reduce the levels of anti-inflammatory cytokines. Free radicals contained in cigarette smoke can provoke cellular destruction and release of intracellular autoantigens. Various toxins found within cigarette smoke may cause DNA damage and genetic mutations, as well as stimulate autoreactive T cells [21]. An alteration in oestrogen metabolism, which has been indicated as a feature in autoimmune disease, has also been found to occur in active smokers [23, 24]. However, the effect of tobacco smoke in the loss of self-tolerance may be more complicated, as it appears that smoking abstinence exacerbates gastrointestinal symptoms in patients with ulcerative colitis (UC). Such an effect has not been noted in patients with Crohn's disease, the other major inflammatory bowel disease.

Silica exposure has also been investigated as a factor involved in autoimmune disease [12, 25, 26]. Silica has been linked with scleroderma, RA, vasculitis and systemic lupus erythematosus (SLE) [12, 25, 26]. Silica acts as an immune stimulant, increasing pro-inflammatory cytokine production, as well as inducing apoptosis and necrosis [12, 25, 26]. Interestingly, silica has also been shown to increase autoantibody production [27] and immune complex formation in animal models. Animals exposed to silica have demonstrated increases in B and CD4 T cell counts, as well as altering T helper and T regulatory cell ratios [28]. Apart from silica, exposure to solvents such as trichloroethylene, mineral spirits, and petroleum based products have been linked to scleroderma and other undifferentiated connective tissue diseases [29].

Primary biliary cirrhosis is one autoimmune disease in which multiple environmental factors have been implicated. Hair dyes have been added to this list, but very few studies address these compounds. Hair dyes have been previously explored in relation to SLE, both epidemiologically and immunologically. This review will examine the literature surrounding the use of hair dyes in SLE in relation to PBC. As PBC and SLE very rarely co-occur in an affected individual, the effect of hair dyes in either disease may help us understand the complex role of these compounds as environmental triggers of autoimmunity.

Primary biliary cirrhosis

PBC is a chronic cholestatic liver disease of autoimmune origin, characterised by inflammatory destruction of the small intrahepatic bile ducts, fibrosis progressing to cirrhosis and subsequent liver failure [30–32]. Many patients are asymptomatic at the time of diagnosis [33–35]. PBC is characterised by anti-mitochondrial (AMA) and disease-specific antinuclear antibody (ANA), which are also found in asymptomatic patients with normal or abnormal biochemical blood tests indicating cholestasis [30, 34, 36–38]. The most common presenting symptoms are those of fatigue, pruritus and arthralgias [30, 34, 39]. More severe presenting symptoms are related to portal hypertension and hepatic decompensation (jaundice, ascites or variceal bleeding), leading to the need for liver transplantation [30, 34, 39]. However, North American and European studies indicate that the percentage of patients with PBC who require liver transplantation has fallen significantly [32]. In general, the course of PBC is slow but unpredictable [30, 34, 35, 39]. Early treatment with urseodeoxycholic acid (UDCA) has greatly improved survival rates and quality of life in PBC patients [30–32]. Whether UDCA is solely anticholestatic or is also immunomodulatory is to be established [30, 32, 40, 41].

PBC is diagnosed on the basis of AMA in serum, biochemical markers of cholestasis, and histological features diagnostic or compatible with PBC [30–32]. Biochemical indices of cholestasis include increased levels of alkaline phosphatase and γGT [30–32]. AMA and disease-specific ANA are usually present at high titres [30, 36–38]. Occasionally, some patients are found to be negative for AMA [36, 37, 42–47]. AMA titres do not appear to correlate with the severity of the disease and do not seem to have any known clinical significance. Disease-specific ANA appear to be able to identify PBC patients with a more aggressive disease [36–38, 44, 48–59]. PBC specific ANA patterns detected by indirect immunofluorescence, include the "multiple nuclear dot" and "nuclear membrane/rim like" patterns [37, 38, 54, 60–62]. The "multiple nuclear dot" pattern is given by antibodies directed against nuclear body proteins such as Sp100, Sp140, promyelocytic leukaemia nuclear body proteins, and small ubiquitin-like modifiers [30, 37, 38, 50, 56, 57, 59, 63–68]. The "nuclear envelope/rim like pattern" corresponds to reactivities specific for gp210 and nucleoporin p62 and other less studied antigens of the nuclear membrane [54]. Up to 50% of PBC patients are positive for these patterns in isolation or in combination, compared to less than 1% of the pathological and healthy controls indicating their significant disease specificity [1, 54, 57]. Immunoglobulin M (IgM) is usually found to be raised. Histological features typical for PBC include destruction of biliary epithelial cells and loss of small bile ducts with portal inflammatory cell infiltration [30–32].

The mechanisms responsible for the induction of autoimmunity are poorly understood. Several articles published in this, as well as previous issues of the journal, are

focusing on the investigation of mechanisms inducing autoimmunity and indeed autoimmune disease [18, 69–83]. The mechanism of molecular mimicry and immunological cross-reactivity between infectious agents or xenobiotics and disease-specific autoantigens has been used to explain the loss of immunological tolerance in autoimmune gastrointestinal and liver diseases, including PBC [84–100].

Several environmental factors have been also implicated in the development of PBC [6, 40, 41, 84, 92, 101–108]. These have included recurrent urinary tract infections [109, 110], xenobiotics [111, 112], and oestrogen deficiency [20, 113] to name a few. Clustering of PBC cases in New York State [114], and the north of England [115] raise further suspicion of environmental factors being involved [6, 104].

Epidemiological studies in PBC and hair dyes use

Epidemiological studies on PBC have demonstrated varying results as to whether hair dye use is a factor in the development of PBC. A French study involving 222 PBC patients and 509 controls was based on a questionnaire of 200 questions covering medical, surgical and reproductive history, demographics and lifestyle [116]. Although exposure to cigarette smoke was found to be associated with PBC, hair dye use was not [116]. In the PBC group, it was found that the average exposures per year was 3.4 (±4.6), which was not significantly different from the control group with the average number of exposures being 3.5 (±4.5) [116]. A much larger study from the US involved 1,032 PBC patients and 1,041 controls matched for sex, age, race and geographical location [20]. The PBC cohort consisted of patients from 23 tertiary care centers for liver disease across the US [20]. Controls were obtained from random-digit dialing of individuals with listed phone numbers [20]. All participants were given a questionnaire by trained personnel, which evaluated social, demographic, personal and family medical histories, lifestyle, and reproductive factors. PBC patients were found to have used hair dyes more frequently than controls (38 times per year compared to 35 times per year) [20]. Nail polish use was also found to be associated with PBC, and of note, the authors indicated that this involved female cases only, which does not appear to be the case with hair dyes [20]. A large epidemiological study from the UK has also found an association between PBC and hair dyes [113]. That study involved two cohorts of PBC patients, with one group consisting of 318 patients in an epidemiological group, and 2,258 patients from a PBC support group [113]. The control group was composed of 2,438 age and sex matched controls. All participants were sent a postal questionnaire on PBC risk factors. An association between hair dye use and PBC was found in both groups of PBC patients. Hair dye use preceded PBC diagnosis in 86% of the epidemiological PBC cases, and 87% of cases from the support group [113]. It should also be noted that 50% of women in all test groups reported previous hair dye use, compared to only 1% of male participants [113].

These epidemiological studies do not appear to analyze the relation of hair dyes with PBC separately in women and men with this disease. In fact, it does not appear that an analysis of hair dye use was limited to females in any epidemiological study of PBC, unlike studies on hair dye use in SLE [117–125]. As well, many epidemiological reports do not indicate the time length of hair dye use, and very few indicate whether hair dye use preceded the diagnosis of PBC.

Hair dyes in SLE

Much like PBC, several pharmacological and chemical materials have been implicated in the development of SLE, including exogenous sex hormones, silica, silicone, solvents, pesticides, mercuric chloride and hair dyes [121]. Hair dyes are of particular interest, as they contain aromatic amines that have been found to induce SLE-like symptoms [123]. An early case study by Freni-Titulaer et al. [119] indicated a positive association between the use of hair dyes and connective tissue disease. A larger study was carried out by Petri and Allbritton [122] involving 218 SLE patients from the Hopkins Lupus Cohort, who were asked to fill in a questionnaire on hair product use. Controls were administered the same questionnaire, and consisted of two groups, the first being 178 first and second degree relatives, and the second being 168 best friends of the patients [122]. That study found no significant relationship between hair dye use and the development of SLE [122]. In a prospective study of 106,391 patients, patients and controls were assessed (based on self-reporting) on hair dye use every 2 years, from 1976 to 1982 [125]. Again, no association was found, even in women with more than 15 years of hair dye use [125]. A longitudinal study by Jimenez-Alonso and colleagues [120] examined whether hair dyes played a role in the prognosis and course of SLE. That study was comprised of 91 SLE and 22 cutaneous lupus patients, divided into non-hair dye users, permanent users, and users but not permanent ones (indicating occasional use, such as bleaching or lowlights) [120]. No significant difference was found in relation to a more severe disease course in the two groups which used hair dyes, and in fact, non-users had more renal involvement than the two user groups [120].

Earlier studies into hair dyes and SLE largely pointed away from hair dyes being involved, however more recent studies have drawn attention back to hair dyes, with an

examination of the immunemodulatory effects of hair dye use. A study by Cooper and colleagues [117] into SLE and hair dye use, subdivided hair dye use into dyes and permanent compounds, as well as limiting their analysis to females, due to the extremely low usage of hair dyes among males. Two hundred and sixty-five SLE patients were obtained from 34 rheumatology practices in Eastern and Southern Carolina, with 355 age, sex, and geographically matched controls obtained through a drivers licence registry [117]. Both smoking and hair dye use were assessed through a 60 min interview. No association between SLE and smoking was found, however, permanent hair dye use was associated with a small increase in the risk of developing SLE, especially if they had used dark coloured dyes [117]. This association increased with a longer duration of use, and there was little evidence of an association with temporary use, or the use of hair straighteners [117]. As ANA is the serological hallmark of SLE, Cooper et al. [118] examined the association between ANA positivity and exposure to environmental toxins, including hair dyes. Serum samples from 266 population-based subjects were obtained, as were occupational and hair dye usage histories [118]. Positivity for ANA was found in 21 (8%) subjects, with an increased prevalence found in those who had been exposed to silica, dust, pesticides and UV radiation, and a smaller association was found with hair dye usage [118].

The immunological effects of hair dye usage have been examined recently in a study by Rubin and colleagues [124], who studied the immune responses in mice repeatedly exposed to *p*-phenylenediamine (PPD), which is a component of many hair dyes. Mice had PPD applied to the dorsum of their ears, which was washed away after 30 min, which was done over several weeks [124]. The same was done for controls, which were exposed to a mixture of olive oil and acetone [124]. Analyses were conducted over several weeks, in which the area of application was histologically examined, and lymph node extracts were analysed by flow cytometry [124]. Increased cellular infiltration was seen after multiple exposures, with the inflammatory response peaking after 4 weeks of exposure to PPD [124]. After 4 weeks, it appeared that the inflammatory response levelled off, indicating that a balance between pro and anti-inflammatory mechanisms had occurred [124]. Flow cytometry demonstrated increased proliferation of both CD4+ and CD8+ T cells in mice exposed to PPD, with the number of Th1 cells increasing with increased numbers of exposure [124]. CD4+Foxp3+ T regulatory (Treg) cells gradually increased up to the fourth week of exposure, at which point they outnumbered the IFNγ and IL-17 producing T cells, and comprised 14% of the total CD4+ population [124]. A second series of analyses were conducted with hair dyes (as opposed to PPD alone), which produced similar results, with the exception that there was a higher Treg increase seen after hair dye exposure [124]. The above studies have demonstrated that hair dye use can induce a pro-inflammatory immune response, as well as possibly influencing the autoantibody profile. It appears also that dye use may indeed lead to the manipulation of suppressor mechanism which depending on the timing may have an anti-inflammatory effect. As well, studies which took into account the higher female preponderance of hair treatment usage, in addition to examining various product types, demonstrated a positive association between SLE and hair product usage.

Hair dyes as a risk for PBC: time to revisit?

Studies examining the association between PBC and hair dyes have been, at best, limited. Several factors addressed in the analysis between hair dye use and SLE, have not been addressed in PBC. First, epidemiological studies do not appear to separate male from female PBC patients, which may lead to an inaccurate prevalence of hair dye use. Accepted risk factors for PBC, such as recurrent urinary tract infections and oestrogen deficiency, have been analysed with the factor of female preponderance being taken into account, but it is not clear as to whether this has been done in regards to hair dyes. As well, they do not appear to define various types of hair dyes, such as dark or light colourings, as well as products used to straighten hair. Nor do they address in detail the length of time that the patients used the hair products for. Refinement of studies to address these issues in SLE demonstrated a positive association between exposure and disease.

Immunological studies have found an increase pro-inflammatory response in mice exposed to PPD and hair dyes, which was followed by an increased regulatory T cell response [124]. An increase in CD4+Foxp3+ Tregs has also been demonstrated in peripheral blood mononuclear cells of PBC patients, also with increased IFNγ [126]. Altered Treg function has been demonstrated to be a feature of PBC, or PBC-like pathology [127–130]. It could be argued that exposure to chemical compounds may induce pro-inflammatory and anti-inflammatory responses, the former prevailing in genetically susceptible individuals, thus contributing to the development of autoimmune disease. It would be of interest to see if any liver pathology is present in animal models of hair dye exposure.

Hair dyes as a risk for advanced PBC: simple questions, straight answers

A significant proportion of patients with PBC are aware of the current research surrounding PBC risk factors.

This information is readily available on the internet, as well as in support groups for PBC patients. As a result, treating physicians should be prepared for questions surrounding the need to take precautions against risk factors, such as repeated hair dye use. In our practice, we ensure that patients are aware that the number of studies performed, as well as the small cohort sizes, prohibits any definitive conclusions from being made. We also make clear to the patient, that the cessation of hair dye use will likely not alter the course of their disease. It needs to be clarified that there are no data to suggest that the continuous exposure to synthetic dyes leads to more advanced disease. We do not encourage patients with PBC to follow any specific instructions, and avoid making any recommendations or advice concerning specific products. Patients with chemical sensitivities or allergies to unspecified chemical compounds are advised to follow conventional precautions applied for everyone. In a culture obsessed with beauty, youth and well-being, changing hair colour has become very common. There is no doubt that hair dye use will continue among men and women despite the potential risks, and it is likely that PBC patients are not the exception.

Conclusion

The list of chemical compounds implicated in the development of autoimmune disease is immense. Hair dyes have demonstrated an association with SLE development, but only after study refinement. It is apparent that when taking into account environmental components in the induction of the disease, care must be given to analyse those results in the context of the study population. Hair dyes are predominantly used by women, and studies must take this into account. As well, the variety of products available must also be addressed, as variability within a compound group may give differing results. These factors are likely to be involved in the limited studies associating hair dye use with PBC. As well, animal models may clarify the immunological mechanisms involved in the process of PBC induction and exposure to chemical compounds.

Conflict of interest None of the authors has a conflict of interest to declare.

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