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A general population from Thailand: incidence of common allergens with emphasis on para-phenylenediamine

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Clinical and Experimental Allergy

Summary

Background Most studies on the prevalence of allergy to the permanent hair dye chemical para-phenylenediamine (PPD) are reported from populations of eczema patients attending patch-test clinics, and are assumed to be much higher than in the normal population. No data exist on incidence of sensitization to PPD resulting from the use of commercial hair dye preparations over a defined time period.

Method A total of 2545 healthy adult volunteers (Bangkok) were screened for PPD allergy through standard patch testing. Volunteers not allergic to PPD were then recruited into two groups: one group applying a commercial hair dye brand as instructed on a monthly basis for 6 months ($n = 548$) and the other group (controls) ($n = 516$) was instructed not to dye their hair for 6 months. Sensitization to PPD resulting from the use of hair dye over this period was then detected by repeat patch testing.

Results The prevalence of PPD allergy in a normal adult population was 2.7% ($m = 2.4\%$, $f = 3.2\%$). Projected to the adult Thai population, at least 1 000 000 Thai individuals could be allergic to PPD. The incidence of sensitization through the monthly application of standard commercial hair dye preparations over a 6-month period was 1.3%, substantially higher than in controls (0.4%), although numbers were small and not statistically significant.

Interpretation There is a higher prevalence of hair dye allergy among the normal population than previously thought. The incidence of new cases of PPD allergy would indicate that current regulations and practice of hair dye exposure lead to PPD sensitization and allergy, which is a public health problem.

Keywords allergic contact dermatitis, chromate, colophonium, formaldehyde, fragrance, hair dye allergy, nickel, normal volunteers, para-phenylenediamine

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Introduction

For over 100 years, para-phenylenediamine (PPD) and other related members of the aromatic amine family have been the main agents used in permanent hair dyes. PPD alone is used in at least 70% of hair dyes [1]. With its low molecular weight (108 Da) and its ability to penetrate the hair shaft and follicle, its strong protein-binding capacity and its rapid polymerization in the presence of a coupler and an oxidizing agent, it is an ideal chemical for hair dyeing. All permanent hair dyes belong to the same aromatic amine family, where there is likely to be a significant degree of immunological cross-reactivity [2]. There is currently no effective alternative to the use of

PPD or other chemically similar aromatic amines in permanent hair dyes.

The same qualities that make it an effective hair dye agent also, however, render it a potent contact allergen. PPD is one of the most potent allergens known; local lymph node assay data indicate it is of similar potency to the classic sensitizer 2,4-dinitro-chlorobenzene [3]. Repeated application of 10% PPD sensitized all 24 subjects in a human maximization test [4]. Sensitization by PPD has, in the past, been considered so great a hazard that its use in hair dyes was banned in Germany, France and Sweden at varying times [5]. To date, most studies on PPD allergy are reported from populations of eczema patients attending a patch test clinic [6–8]. Such patients are screened

with the 'standard' series of common contact allergens, which would include PPD. Limited population studies have shown a much higher rate of contact allergy in patch test populations than in estimates of the general public [9].

Although the majority of adult women in typical industrialized countries use hair dye [10], knowledge of the scale and degree of hair dye allergy among the normal population is sparse [11, 12] and an accurate assessment of incidence of new cases is completely unknown. We report the first prevalence and incidence study of PPD allergy among a large, healthy population.

Methods

Subjects

Phase 1. Healthy Thai adults aged 18–55 years of age were recruited from the Bangkok area ($n = 2545$) via an advertisement in the local press. Entry criteria are listed in the Appendix A, in particular hair dyeing practice. Important exclusion criteria included skin disease (including scalp involvement). All individuals gave fully informed, written consent. The study received local ethical committee approval, situated at the Institute of Dermatology, Bangkok, Thailand (May 2004). Recruitment was from all socio-economic groups and there was no limitation of either hair style or hair type (straight/wavy/permed/straightened).

Testing for PPD allergy/sensitization was performed by conventional means through skin patch testing. Diagnostic patch test materials (1% PPD in petrolatum) were applied in Finn[®] chambers (Epitest Oy, Tuusula, Finland) using Scanpor[®] (Beiersdorf, Hamburg, Germany) adhesive tape on the upper back. Patches were removed after 48 h and sites inspected for positive reactions approximately 1 h after patch test removal, according to the standard ICDRG criteria [13].

Besides screening for PPD allergy, subjects were screened for other common contact allergens at standard test concentrations – nickel 5% pet. (a metal found in low-carat gold and in fasteners in clothing) ($n = 1397$); formaldehyde 1% aqueous ($n = 2545$); methylchloroisothiazolinone/methylisothiazolinone (MCI/MI) 0.01% aqueous ($n = 1178$) (commonly used as preservative agents in toiletries and household products); potassium dichromate 0.5% pet. (found chiefly in leather as a tanning agent and in wet plaster/cement) ($n = 1397$); colophonium (pine-resin extract in plasters) ($n = 1178$) and the common fragrance allergens isoeugenol, evernia prunastri and myroxylon pereirae ($n = 2545$). All patch test materials were obtained from Chemotechnique Diagnostics, Vellinge, Sweden. Standard patch test reading technique was used [13]. The differences in the numbers of subjects patch tested to these various allergens relate to an attempt by the investigators to minimize the total number of patches

placed (with potentially uncomfortable positive tests) in each individual volunteer.

Phase 2. Approximately half of the volunteers were recruited from the phase 1 study, such that each group started with approximately 620 individuals. Subjects were matched by criteria of sex, age and hair dyeing practice to populate the two arms of the study. All subjects who were found to be allergic through patch testing to PPD were excluded. There was no limitation of either hair style or hair type (straight/wavy/permed/straightened). All hair length was allowed, except extremely short or shorn styles (see appendix A).

The hair dye group ($n = 548$) used a permanent hair dye to give a black hair colouring (one of two commercially available market leaders, with PPD concentration approximately 1.5%) according to the general use instructions, but in an unsupervised manner. They used permanent hair dye (approximately 30 min exposure) once per month (six exposures in total). There were 195 males and 353 females in this group. Age was broken down into the following ranges with percentages of 548 given in brackets: 18–24 years (28%); 25–29 years (13%); 30–35 years (17%); 36–40 years (14%) and 41–55 years (28%). There were a number of subjects who were lost to follow-up during the course of the study group (approximately 70 from the hair dye group and 100 from the control group. The figures given in brackets were calculated after those lost to follow-up were excluded from the analysis.

The control group ($n = 516$) was asked not to use any hair dye agent over the 6-month study period. They had been perfectly matched to the hair dye group; however, this became less of a perfect match after some individuals were lost to follow-up, although they were similar to the whole group in terms of gender and age.

Both groups were re-tested for PPD allergy at the end of the study, to detect any sensitization to PPD resulting from the use of hair dye over this study period.

Statistical analysis

A confidence interval for the binomial proportion of normal volunteers who were sensitized to PPD was estimated using the standard formula with normal approximation. Fisher's exact test was used to determine if the proportion sensitized in the permanent hair dye group was different from that sensitized in the control group and also to test if the proportion of normal volunteers testing positive to PPD was different from the proportion observed in the patch test clinic.

Given that the groups were similarly distributed in terms of age and sex and given that the two groups had readings at 48 h, we feel that the groups can be compared statistically. For the various allergens, a χ^2 test was used to

Table 1. Prevalence of contact allergens in a normal Thai population

	Total (%)	Male (%)	Female (%)	18–29 years (%*)	30–40 years (%*)	41–55 year (%*)
PPD	69 (2.7)	20 (2.4)	49 (3.2)	13 (1.3)	22 (3.0)	34 (3.4)
Nickel	389 (27.8)	55 (3.9)	334 (33.8)	195 (34.9)	120 (27.7)	74 (19.0)
Fragrance	63 (2.5)	21 (2.1)	42 (2.7)	16 (1.6)	15 (2.0)	32 (3.4)
Formaldehyde	19 (0.7)	8 (0.8)	11 (0.7)	7 (0.9)	10 (1.4)	2 (0.3)
MCI/MI	14 (1.2)	7 (1.2)	7 (1.2)	2 (0.4)	6 (1.6)	6 (1.8)
Colophonium	24 (2.0)	8 (1.4)	16 (2.7)	7 (1.5)	10 (2.8)	7 (2.1)
Chromate	37 (2.6)	9 (2.2)	28 (2.8)	21 (1.5)	10 (2.8)	6 (2.1)

For PPD, fragrance and formaldehyde: $n = 2545$; $m = 980$; $f = 1565$; age range 18–55.

For nickel and chromate: $n = 1397$; $m = 410$; $f = 987$; age range 18–55.

For colophonium and MCI/MI: $n = 1178$; $m = 570$; $f = 578$; age range 18–55.

*Expressed percentage of that age group.

PPD, para-phenylenediamine; MCI/MI, methylchloroisothiazolinone/methylisothiazolinone.

check for any differences in the proportions sensitized between the different age groups. A Cochran–Armitage trend test was used to break this down further, and determine if the differences in proportions over age could be attributed to a linear response.

All tests were performed using Proc Freq in SAS [14].

Results

Phase 1

The results for prevalence of contact allergens in a normal Thai population are given in Table 1. The total percentage of normal subjects allergic to PPD was surprisingly high at 2.7%. The female/male ratio was 4:3. There was a significant rise in prevalence with increased age ($P < 0.01$).

According to the most recent census there are 24 446 737 adult Thai males and 25 375 500 females [15]. Using these data and extrapolating from our sample, we estimate that there are at least 1 000 000 Thai adults allergic to PPD.

Among other common contact allergens, contact allergy to nickel was exceptionally high with a third of the female population positive to nickel on allergy testing. Prevalence of nickel allergy was highest in the younger age group and significantly decreased with age ($P < 0.001$). Our fragrance markers (which may be classified as cosmetic consumer allergens), like PPD, had a significant increase in prevalence with age, whereas MCI/MI did not reach statistical significance (fragrance $P < 0.05$ and MCI/MI $P < 0.2$).

To assess how our prevalence data in normal volunteers compared with patch test clinic data from Bangkok, we used previously published data [16] (Table 2). Our data show that PPD allergy rate in the normal population is approximately half that of the rate in the contact dermatitis clinic ($P = 0.01$). In contrast there is bigger discrepancy

Table 2. Comparison of patch test reactions in normal volunteers and patch test populations

	Normal volunteers (%) $n = 2545$	Data from patch test clinic (%) $n = 642$
PPD	2.7	4.6
Nickel*	27.8	27.2
Fragrance	2.5	15.5
Formaldehyde	0.7	4.5
Chromate*	2.6	13.9
Colophonium†	2.1	6.7

* $n = 1397$; † $n = 1178$

There is a statistically significant difference in incidence rates of these allergens in subjects recruited in patch test clinics compared with normal volunteers ($P < 0.025$). There were no data for MCI/MI from Thai contact clinics.

PPD, para-phenylenediamine; MCI/MI, methylchloroisothiazolinone/methylisothiazolinone.

pancy between normal volunteer population and contact dermatitis clinic with formaldehyde and fragrance (both approximately a sixfold difference) and chromate (approximately a fivefold difference). Nickel allergy rates were very similar in both groups.

Phase 2

Two of the hair dye-user group and 34 of the control group failed to reach the end of the study, the main reason being subjects reverting to dyeing of hair in the control group.

Seven out of 548 (1.3%) of the hair dye user group was PPD positive; 2/516 (0.4%) of the control group were PPD positive on patch testing (Table 3). This difference did not reach statistical significance on analysis with Fisher's exact test ($P = 0.10$). Virtually all the reactions were grade 1.

Table 3. Prospective study of PPD sensitization after 6 months of routine hair dyeing procedure

Group	Total	Sensitized (after 6 months)
(1) Dyes hair	548	7 (1.3%)
(2) Control	516	2 (0.4%)

PPD, para-phenylenediamine.

Discussion

The prevalence of PPD allergy in our normal Thai population was surprisingly high (Table 1). It is probably an underestimation as, due to logistical reasons, only one reading of the patch test site to look for a reaction was performed, at 48 h. Previous research indicates a further 33% of allergy cases would be detected with a second later reading at 4 days [16]. Alternatively, the elicitation threshold may merely have altered in the intervening time, a possibility we consider unlikely. In previous studies among 593 young Italian male military recruits, the rate of PPD patch testing was, at 0.5%, unsurprisingly very low [11]. A study of a smaller group of normal German volunteers gave a prevalence of 1.5% ($m = 1.0\%$, $f = 2.0\%$, m/f ratio = 1/2) [12]. The higher prevalence in our study may be explained by the use of darker dyes in Thailand (dyes for lighter hair colours usually have a lower concentration of PPD). The increased male/female ratio seen is a reflection of greater male use of hair dye in Asia, whereas with patch test data from European studies, a male:female ratio of 1 : 2 is usually seen. This ratio is much more equal, or even reversed, in patch test data from Asia [17]. There was a significantly higher incidence in the older age group. A number of factors could contribute to this. They were probably exposed to higher levels of PPD in order to achieve more effective coverage of grey hair. However, prevalence data from patch test clinics on cosmetic allergens such as fragrance show a gradually increasing frequency with progressing age, suggesting that repeated, frequent exposure to a contact allergen at low doses over the course of years will result in new cases of sensitization [18].

We estimate from our data that a million Thai adults are sensitized to PPD. However, they may not necessarily be symptomatic, as elicitation studies have shown that both time and dose of PPD exposure are crucial factors in producing a skin reaction [19, 20]; yet even if only a small proportion of these patients develop overt disease, this constitutes a major public health problem. One percent PPD applied for 30 min (a standard time for hair dye application) will only produce an elicitation reaction in 50% of subjects sensitized to PPD [20]. Subjects applying lighter shades of dye (with lower concentration of PPD) and leaving it on for shorter time periods are less likely to

produce an inflammatory reaction. It is a common observation that clinically significant hair dye reactions often occur after the dye has been left on for longer or a darker shade used, which is different from the patient's usual practice. We have previously reported that many subjects who have developed hair dye allergy persist in dyeing their hair [21], presumably due to cultural need. Such methods as reducing exposure time are used to achieve this. Repeated exposure to low doses of PPD in already sensitized subjects should increase the allergic response in time, but this has not been studied to date in humans, though there is suggestive data in animal models [22].

We have also reported on the prevalence of sensitization to other common contact allergens. Nickel allergy was present in a third of all females tested, and significantly more common in the younger age group. Ear piercing (and the total number of piercings) is the single most important predictive factor in developing nickel sensitization [23]. One reason it may be more common in younger females is an increased frequency of multiple ear piercings in that group. An alternative or additional cause may be high nickel levels in 'posts' left in the ear after piercing in comparison with previous decades. The EU has already legislated to limit the level of nickel in jewellery. The high level of nickel sensitization in Thailand would appear to be an additional Public Health issue. Cement exposure has traditionally been considered the main cause for chromate sensitization but most authorities agree that 'cement dermatitis' is decreasing in frequency [24]. Tanned leather is an important source of chromate exposure and may explain the higher frequency of chromate sensitization in the young female group. There was also a high level of fragrance sensitization in the normal population; as with data from patch test clinics it is more common in females [25] and there is progressive increase with age [18].

It is often claimed that contact allergy prevalence data from patch test clinics vastly overrate the true prevalence in the normal population. However, our comparison (Table 2) shows that PPD sensitization rate in the normal population is approximately half that of the rate reported in a Bangkok patch test clinic. This would mean that while half the cases of PPD allergy detected in patch test clinics may be the primary cause of referral, the other half may be detected coincidentally with the use of the standard screening of common contact allergens ('the standard series'). In contrast there is a bigger discrepancy between normal volunteer population and patch test clinic data with regard to formaldehyde, fragrance and chromate allergy. This may be because the allergic cause of the dermatitis may be difficult to identify and so patch testing is frequently requested (formaldehyde), the allergy may be a frequent secondary problem in different dermatitis cases [18] and a high occupational aetiology leading to higher

rates of referral (potassium dichromate). There is an equal prevalence of nickel sensitization in both normal and patch test clinic populations.

In the phase 2 study, all volunteers were PPD patch test negative at the original screening. In the control group who did not dye their hair for 6 months, 2/516 (0.4%) reacted to PPD on repeat testing after 6 months. One of these may have been due to non-compliance (i.e. continued use of hair dye). The other positive could represent active sensitization from the first PPD screen, which can occur [26]; however, our data from this study confirm that this phenomenon is very uncommon. The permanent hair dye user group displayed a sensitization rate of 7/548 (1.3%). Incorporating the control group results, the best estimate of sensitization to monthly use of a standard, commercially available permanent hair dye over 6-months was 0.9%. Only 1 out of the 7 had a clinically severe reaction during the 6 month study period—it would appear that in the majority of subjects PPD sensitization from hair dye exposure does not initially lead to severe elicitation reactions, but this can occur as repeated exposure increases the level of sensitization.

Studies of elicitation dose response relationships have become more complex in order to replicate real-life exposures [27, 28]. Such investigations should help considerably in the secondary prevention of allergic contact dermatitis. However, it is more important to prevent the induction of skin sensitization in the first place, i.e. primary prevention. Experimental investigation of human sensitization to contact allergens has been limited, not least for ethical reasons, to relatively simple types of study [4]. In the present work the study was carefully controlled from many perspectives but allowed the volunteers to use the hair dye formulations according to the supplied instructions. Thus the study was designed to incorporate normal use of a consumer product (permanent hair dye) containing the contact allergen PPD. Existing legislation allows consumer hair dyes to contain up to 6% PPD (3% when added to the oxidizing solution required to develop the colour). All leading consumer brands of hair dyes use PPD well within this level; the ones used in this study were approximately 1.5%. However, even with these levels, 1% of users became sensitized to PPD over the course of 6 months.

There is a strong cultural demand for the use of hair dyes; the medical profession may be alone in having white hair perceived as an advantage at work. There is extremely strong resistance to stop dyeing hair, even when the patient has been told they are allergic to it and they run the risk of having severe reactions [21]. A recent survey from Denmark revealed among the normal adult population that 75% of women and 18% of men have used hair dye [10]. Furthermore, the median age of first use of hair dye for both males and females was in the teenage years [10]. It would appear that more people are dyeing their

hair and at a younger age. The use of hair dye is changing from not only hiding white hair but also to being used for style and fashion. Dyeing hair is such an accepted part of everyday life that people will clearly not stop using it. Urgent work is needed to establish safer use patterns for hair dye chemicals as well as to identify safer hair dye chemicals.

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Appendix A: Entry criteria for this study:

Males and females, aged 18–55 years old (matching in each cell)

Live permanently in test area (Greater Bangkok)

All social classes (A/B/C/D/E SES), matching in each cell (based on monthly HH income):

A = Bht. 50 000 or higher, B = Bht. 40 000–49 999, C = Bht. 20 000–39 999, D = Bht. 10 000–19 999 and E = Bht. 9 999 or lower

No limitation of hair style and hair type (straight/wavy/perm/straightened)

All hair length, except for extremely short hair – skin head

Excluding those who describe themselves as 'normally sensitive to product reaction' or 'have pre-existing scalp conditions'

(i.e. excessive hair loss, severe dandruff, scalp wounds)

Colourant and non-colourant users (matching in each cell)

Colourant users – not using colourant product within the past 2 months

Non-colourant users – never used colourant product or use for longer than 24 months

Have or have no grey hair, but being non-rejecters of hair dye and black colourant

Apart from those provided for the test, the following are not permitted during the test period (any usage must be informed to

Research Dynamics):

No usage of other colourant products

No hair perming or straightening

No major hair treatment or major haircut

Willing to join the test for at least a 6-month period, willing to use the tested product, following instructions and willing to be patch tested – all are required to give informed, written consent