ORIGINAL ARTICLE

The Value of Adding 1,3-Diphenylguanidine, N-Cyclohexyl-N-Phenyl-4-Phenylenediamine, N-Cyclohexylthiophthalimide and *Ylang-ylang oil* to the European Baseline Series to Improve the Detection of Rubber and Fragrance Allergy Among Patients Suspected with Contact Dermatitis in Hospital Selayang

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Abstract

Introduction

Allergic contact dermatitis affects 15 to 20% of the population. The pattern of contact allergy varies across nations. Therefore, many countries utilize their unique individual baseline series for patch testing. In this study, we aimed to assess the outcome of rubber and fragrance allergy detection with the addition of 1,3-Diphenylguanidine. N-Cyclohexyl-N-Phenyl-4-Phenylenediamine, N-Cyclohexylthiophthalimide and *Ylang ylang oil.*

Methods

This a cross-sectional study on 292 patients who underwent patch testing with European Baseline Series, 3 additional rubber allergens namely 1,3-Diphenylguanidine, N-Cyclohexyl-N-Phenyl-4-Phenylenediamine, N-Cyclohexylthiophthalimide and *Ylang ylang oil* between July 2015 to December 2016. Additional patch test series were also added based on relevant clinical history. The patch test reactions were read at 48 and 96 hours after application of the allergens.

Results

A total of 292 patients completed the study. There were 118 (40.4%) males and 174 (59.6%) females. The mean age was 43 years old (range 19 to 78 years). Two-third of patients had atopy. The sensitization rate increased to 70.5% from 67.1% with the four additional allergens included in the European Baseline Series. The rubber allergy detection rate significantly increased by 53.8% ($p<0.00001$) with the addition of 1,3-Diphenylguanidine, N-Cyclohexyl-N-Phenyl-4-Phenylenediamine, and N-Cyclohexylthiophthalimide to the European Baseline Series. The addition of 1,3-Diphenylguanidine alone to the baseline series improved the rubber allergy detection rate by 49.2%. The sensitization rate of 1,3-Diphenylguanidine was 10.6%, which ranked the third most sensitizing allergen in the cohort. The addition of N-Cyclohexyl-N-Phenyl-4-Phenylenediamine and N-Cyclohexylthiophthalimide only increased the detection rate of rubber allergy by 4.6%, where each had a sensitization rate of 1.4%. Fragrance allergy detection rate increased by 6.5% with the addition of *Ylang ylang oil* to the European Baseline Series. The sensitization rate of *Ylang ylang oil* was 3.4% in this cohort..

Conclusion

The overall detection rate of sensitization increased by 3.4% with the addition of 1,3-Diphenylguanidine, N-Cyclohexyl-N-Phenyl-4-Phenylenediamine, N-Cyclohexylthiophthalimide and *Ylang ylang oil.* The addition of 1,3-Diphenylguanidine rubber in the baseline series improved the detection of rubber sensitization by 49.2% while the addition of *Ylang ylang oil* increased fragrance sensitization detection rate by 6.5%.

Key words: *Allergic contact dermatitis, 1,3-Diphenylguanidine, N-Cyclohexyl-N-Phenyl-4-Phenylenediamine, N-Cyclohexylthiophthalimide, Ylang ylang oil*

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Introduction

Allergic contact dermatitis affects 15 to 20% of the population in Europe. In United States, the point prevalence of allergic contact dermatitis is 15.2% in teenagers and 18.6% in adults.[1] The common causative allergens vary between nations due to the industrial policies and cultural practice. Thus many countries constructed their individual baseline series for patch test.

In a retrospective analysis of local patch test data[2], four emerging allergens were identified from the rubber additives series and fragrance series - 1,3-Diphenylguanidine, N-Cyclohexyl-N-Phenyl-4-Phenylenediamine, N-Cyclohexylthiophthalimide and *Ylang ylang oil* with the sensitization rates of 11.1%, 3.9%, 3.6% and 14.6%.[2] The additional patch test series were tested on selected patients whom were clinically suspected with rubber or fragrance allergy.

In this study, we incorporated these 4 allergens into the European Baseline Series with the aim to assess the outcome of the overall contact sensitization rate as well as the detection rate of rubber and fragrance allergy.

Materials and Methods

A cross-sectional study was performed on selected patients aged 18 and above suspected with allergic contact dermatitis in Selayang Hospital between June 2015 and December 2017. We excluded patients with active dermatitis, excessive sun exposure, topical corticosteroid application on the test site within one week prior to the study, ingestion of oral prednisolone (exceeding 20mg daily) or other immunosuppressant agents, pregnant or lactating ladies, and those with previous history of patch test.

All candidates underwent patch test using European Baseline Series with the addition of 1,3-Diphenylguanidine, N-Cyclohexyl-N-Phenyl-4-Phenylenediamine, N-Cyclohexylthiophthalimide and *Ylang ylang oil.* Additional patch test series were also added based on individual presentation as part of the daily routine practice.

The European Baseline Series from Chemotechnique Diagnostics in year 2015 consisted of 30 allergens:

potassium dichromate, p-phenylenediamine (PPD), thiuram mix, neomycin sulfate, cobalt (II) chloride hexahydrate, benzocaine, nickel (II) sulfate hexahydrate, clioquinol, colophonium, paraben mix, N-Isopropyl-N-Phenyl-4-Phenylenediamine (IPPD), lanolin alcohol, mercapto mix, epoxy resin, balsam of peru, 4-Tert-Butylphenol formaldehyde resin (PTBP), 2-merceptobenzothiazole (MBT), formaldehyde, fragrance mix I, sesquiterpene lactone mix, quaternium 15, propolis, methylisothiazolinone /methylchloroisothiazolinone, budesonide, tixocortol-21-pivalate, methyldibromo glutaronitrile, fragrance mix II, lyral, methylisothiazolinone and textile dye mix.

The patch test reactions were read at 48 and 96 hours after application of the allergens. Interpretation of patch test reactions were based on the reading criteria of International Contact Dermatitis Research Group (ICDRG) guidelines.

Data was analyzed using IBM SPSS® Statistics version 23.

Results

A total of 292 patients, comprising of 118 males (40.4%) and 174 females (59.6%) aged 18 years and above with the mean age of 43 years, were patch tested during the 18-month study period (Table 1). Among the subjects, 157 (53.8%) were Malay, 96 (32.9%) were Chinese, 37 (12.7%) were Indian and 2 (0.7%) of other races. Two-third of the patients had a background history of atopy.

Table 1. Patient Demographic and Characteristics

Characteristics	n=292 (%)
Age, median (IQR)	41 (28, 57)
Gender	
Male	118 (40.4)
Female	174 (59.6)
Ethnicity	
Malay	157 (53.8)
Chinese	96 (32.9)
Indian	37 (12.7)
Others	2 (0.7)
Atopy	
Presence	200 (68.5)
Absence	92 (31.5)

When tested with European Baseline Series, 67.1% of patients developed at least 1 positive reaction to the patch test allergens. The detected sensitization rate increased to 70.5% with the 4 additional allergens. The difference was not statistically significant (p=0.37). The top ten common allergens were nickel sulfate (22.9%), textile dye mix (13%), 1,3-Diphenylguanidine (10.6%), fragrance mix I (9.9%), methylisothiazolinone (7.9%), cobalt chloride (6.8%), balsam of Peru (6.8%), paraben mix (6.5%), formaldehyde (5.5%), and potassium dichromate (5.5%) (Table 2).

Table 2. Top 10 common allergens in the modified European Baseline Series in Hospital Selayang between July 2015 and December 2016

Allergen	Frequency of Tested Positive, n=292 (%)
Nickel Sulfate	67 (22.9)
Textile Dye Mix	38 (13.0)
1,3 Diphenylguanidine	31 (10.6)
Fragrance Mix I	29 (9.9)
Methylisothiazolinone	23 (7.9)
Cobalt Chloride	20 (6.8)
Balsam of Peru	20 (6.8)
Paraben Mix	19 (6.5)
Formaldehyde	16 (5.5)
Potassium Dichromate	16 (5.5)

Table 3. Sensitization rate of rubber allergens in the European Baseline Series

Rubber Allergen	Frequency of Tested Positive, n=292 (%)
Thiuram Mix	8 (2.7)
2-Mercaptobenzothiazole	4 (1.4)
Mercapto Mix	2 (0.7)
N-Isopropyl-N-Phenyl-4-Phenylenediamine	1 (0.3)

Table 4. Sensitization rate of fragrance allergens in the European Baseline Series

Allergen	Frequency of Tested Positive, n=292 (%)
Fragrance Mix I	29 (9.9)
Balsam of Peru	20 (6.8)
Fragrance Mix II	5 (1.7)
Lyral	2 (0.7)

Compared to the original European Baseline Series (EBS) with 3 rubber allergens, the modified baseline series detected a significantly higher number rubber allergy (p<0.0001). Among the EBS rubber allergens, thiuram mix (2.7%) had the highest sensitization rate, followed by 2-mercaptobenzothiazole (1.4%), mercapto mix (0.7%) and N-Isopropyl-N-Phenyl-4-Phenylenediamine (Table 3). The sensitivity of the combination of EBS with each individual additional rubber allergen was also analyzed. When compared to using EBS alone, the combination with 1,3-diphenylguanidine significantly raised the sensitivity of detecting rubber allergy by 49.2% (p<0.00001). Combination with either N-Cyclohexyl-N-Phenyl-4-Phenylenediamine or N-(Cyclohexylthio) phthalimide, did not contribute to significant improvement in the patch test results (p=0.545) (Table 5).

Table 5. The sensitization rate of European Baseline Series rubber allergens and its combination with additional rubber allergens

Test Allergens	Detection Rate, n (%)	*p* value
European Baseline Series Rubber Allergens Alone	15 (23.1)	-
European Baseline Series Rubber Allergens + 3 Additional Rubber Allergens	50 (76.9)	*<0.00001*
European Baseline Series Rubber Allergens + 1,3 Diphenylguanidine	47 (72.3)	*<0.00001*
European Baseline Series Rubber Allergens + N-Cyclohexyl-N-Phenyl-4-Phenylenediamine	18 (27.7)	*0.545*
European Baseline Series Rubber Allergens + N-(Cyclohexylthio) phthalimide	18 (27.7)	*0.545*

Fragrance mix I (9.9%) was the most common fragrance allergen in the EBS (Table 4). The 4 fragrance allergens in the EBS (balsam of Peru, fragrance mix I, fragrance mix II, lyral) detected 62.3% of the fragrance allergy cases. A further detection of 6.5% of cases were achieved with the addition of *Ylang ylang oil* though the difference was not significant (p=0.499).

There were 5 allergens in the EBS with low sensitization rates in our cohort. Lyral, mercapto mix and primin were each tested positive in 0.7% of the subjects. N-Isopropyl-N-Phenyl-4-Phenylenediamine and budesonide only showed 0.3% positive reactions.

Discussion

Historical Comparison

A historical comparison was made with a retrospective study from the same centre, Hospital Selayang. It studied the contact sensitization rate of 705 subjects between 2011 and 2013 (36 months) (Table 6). The overall sensitization rate of EBS in the study was almost similar to the current study (66.1% vs 67.1%). There was a reduction in the rate of positive reaction to N-Cyclohexyl-N-Phenyl-4-Phenylenediamine and *Ylang ylang oil.* This could be explained by patient selection to undergo patch test with extended series. They were strongly suspected to have allergic contact dermatitis to rubber or fragrance based on exposure history and were then tested with rubber or fragrance series. Hence, the subjects were not a random population. When the allergens were tested on all patients in our study, the sensitization rates were significantly reduced except for 1,3-diphenylguanidine and N-Cyclohexylthiophthalimide. The sensitizing rate of 1,3-Diphenylguanidine was hugging around 10%. The sensitizing rate of N-Cyclohexylthiophthalimide was 1.4% (decreased from 3.6%).

Bendewald et al. carried out an 8-year retrospective review on patch test using rubber allergens.[3] The positive rate of reaction to diphenylguanidine in this study was 7.5%. Bendewald et al also made a comparison with 3 other similar studies which showed a prevalence of diphenylguanidine allergy of 1% to 4.4%.[4,5,6] The rates of sensitisation to N-(Cyclohexylthio) Phthalimide (5.2%) and N-Cyclohexyl-N-Phenyl-4-Phenylenediamine (1.3%) were comparable to our data. The study also demonstrated that carba mix had a low sensitivity in detecting diphenylguanidine allergy. 1,3-Diphenylguanidine is a rubber accelerator which is also a component within carba mix. The prevalence of diphenylguanidine allergy is gradually rising. Most cases have been reported with exposure to synthetic gloves.[7]

Ylang ylang oil, well known for its fragrant scent, is a plant found in South East Asian countries such as in Malaysia, Indonesia, Vietnam and some islands of Indian Ocean. The essential oil extracted from the flower is widely used in aromatherapy, perfume and food industry. The plant is also used by traditional healers in many aspects, such as treatment of asthma, malaria, depression, itch, gout and headache.[8] *Ylang ylang oil* has been included within the North American baseline screening series since 2001.[9] Prior to that, a multicenter study on fragrance contact dermatitis involving centers from Japan, Northern Ireland, United States, Sweden, England and Switzerland showed that the addition of *Ylang ylang oil*, narcissus oil, and sandalwood oil to fragrance mix would detect 94.2% of the cases of fragrance contact dermatitis.[10] In this study, the sensitization rate to *Ylang ylang oil* was 17.4%, which was the third most common fragrance allergen. *Ylang ylang oil* is also present in the China baseline series. Studies which have included *Ylang ylang oil* in the routine testing of patients with contact dermatitis documented prevalence rates between 0.7% and 2.6%.[11,12] Our current study reported a sensitization rate of 3.4% with *Ylang ylang oil* was comparable to other parts of the world. The previous reported 14.6% in the same center reflected a patient selection bias instead of a true increase of *Ylang ylang oil* allergy. The addition of *Ylang ylang oil* in the EBS in our cohort improved the fragrance allergy detection rate from 62.3% to 68.8%.

Table 6. Comparison between 2 study findings in Hospital Selayang

		2011-2013	2015-2016	*p*
Duration of study period in months		36	18	-
Total number of subjects patch tested		705	292	-
Rate of positive reaction European Baseline Series		466 (66.1%)	196 (67.1%)	*0.755*
Rate of positive reaction European Baseline Series + Additional Series		546 (77.4%)	229 (78.4%)	*0.736*
Rate of positive reaction to specific rubber allergen [in the total number of patients tested with rubber series (2011-2013) or modified European Baseline Series (2015-2016)]	1,3 Diphenylguanidine	52 (11.1%) n=468	31 (10.6%) ↓ n=292	*0.824*
	N-Cyclohexyl-N-Phenyl-4-Phenylene-Diamine	18 (3.9%) n=462	4 (1.4%) ↓ n=292	*0.047*
	N-Cyclohexylthio phthalimide	17 (3.6%) n=472	4 (1.4%) ↓ n=292	*0.064*
Rate of positive reaction to *Ylang ylang oil* (in total number of patients tested with *Ylang ylang oil*)		21 (14.6%) n=144	10 (3.4%) ↓ n=292	*0.00002*

As seen in the results section (Table 5), incorporating all 4 additional allergens in the baseline series did improve the overall contact sensitization rate. The detection of rubber allergy was significantly improved with the presence of 1,3-Diphenylguanidine. Therefore, 1,3-Diphenylguanidine should be considered to be included in the baseline screening series in our population. We are yet to formulate our very own local baseline series which could detect at least 90% of fragrance and rubber contact dermatitis. A multicenter study involving a larger local population may provide a better reflection of the local pattern of contact sensitization. Based on the current data, there are other allergens which are locally important but yet to be identified to improve further the contact sensitization detection rate.

Comparison of Data With Other Countries

Studies published from the local patch test data in Singapore[13], Thailand[14], Hong Kong[15], and Korea[16], United Kingdom[12] and North America[17] were reviewed (Table 7). Females seemed to have a higher preponderance to develop contact dermatitis in all nations. The sensitization rates were as follow: Korea (85.8%), Thailand (73.8%), our study (70.5%), North America (63.8%), Singapore (47.1%) and the United Kingdom (43%).

Nickel sulfate remained the most common sensitizing allergen in all listed centres. Its presence in jewellery, cosmetics, household products, coins, and food explain the high prevalence of nickel sulfate allergy.[18] The European Union Nickel Directive regulation in 1994 had led to a temporary reduction in nickel allergy. However the improvement was not sustained in further analysis thereafter. Non-compliance to the legislation was a raised concern.[19]

Fragrance allergens were the next most common allergens across the countries, namely fragrance mix I and balsam of Peru. A Swedish study demonstrated that 88% out of 204 products (shampoos, hair conditioners, liquid soaps, wet tissues, washing-up liquids, and multipurpose cleansers) analysed contained sensitizing fragrance allergens.[20] Fragrance mix II and lyral were included in the EBS in 2008. The recommendation was based on the reported allergy rates of up to 5% to Fragrance mix II and between 1-3% to lyral in various centres in the Europe. The prevalence of lyral allergy in North America however was only 0.4%. The difference was attributed to the higher concentrations in the EU deodorants.[21] Fragrance mix II was among the top 10 common allergens in United Kingdom (3.2%), Singapore (3.9%) and North America (5.2%). In our study, the sensitization rate of Fragrance mix II and lyral were 1.7% and 0.7% respectively. Among the other countries, *Ylang ylang oil* was only present in the North American baseline series, and it was not among the top 20 common allergens list.

Apart from nickel sulfate and fragrance allergens, cobalt chloride, formaldehyde, neomycin sulfate, thiuram mix, para-phenylenediamne, paraben mix, methyl-chloroisothiazolinone and methylisothiazolinone were among the top 10 allergens in these studies. Besides textile dye mix (which was added to the EBS in 2015) and 1,3-Diphenylguanidine (which was not routinely tested in the other countries except for North America), the rest of the common allergens were comparable to the mentioned centres.

Rubber gloves usage among healthcare, industrial and domestic workers resulted in the increasing trend of rubber allergy. Latex allergy was a major concern due to the occurrence of immediate hypersensitivity reaction. Subsequently, synthetic rubber gloves gradually replaced natural rubber latex gloves. However, the rubber accelerators used in the vulcanization process such as thiuram and carbamates also induced allergic contact

dermatitis. Thiuram allergy seemed to have declined followed by a rise in sensitization to carbamates. 1,3-Diphenylguanidine, also a carbamate, is increasingly identified as a clinically significant sensitizing agent.[22,23,24] This phenomenon is probably related to the change in manufacturing ingredients. In a previous local study carried out between 1994 and 1996, Rohna et al demonstrated that thiuram was the most common sensitizing rubber allergen, while 1,3-Diphenylguanidine was less common.[25] In contrast, the results in our study showed that we have more patients sensitized to 1,3-Diphenylguanidine.

An allergen eligible for inclusion in the screening series should have a sensitization rate of 0.5% to 1%, when routinely tested on patients with suspected contact allergy.[26] In the current study, both budesonide and IPPD had only 0.3% positive reactions. IPPD is one of the components of black rubber mix, which functions as an antidegradant in the process of rubber manufacturing. Lam et al. and Waranya et al. reported a prevalence of 0.4% and 1.6% in Hong Kong and Thailand respectively.[26,27] Other centers utilized black rubber mix in the screening series instead of IPPD alone. In the previous study in Hospital Selayang performed between 2011 and 2013, the rate of positive reaction to IPPD was 3.8%. Our low detection rate could possibly be attributed to the change of ingredients in products.

Budesonide, a class B corticosteroid in the European Baseline Series is used as a marker to reflect corticosteroid allergy of the same class. Other class B corticosteroids include amcinonide, desonide, fluocinolone acetonide, halcinonide, triamcinolone (acetonide, diacetate), oral budesonide and oral triamcinolone. Budesonide also cross reacts with class D corticosteroids.[28] The studies performed in different continents have demonstrated a prevalence between 0.3% to 2% for budesonide (Ochi 2017, Dararattanaroj 2016, Yu DS 2016, Toholka 2015, Warshaw 2015).[13,14,16,17,29] Ochi et al. and Dararattanaroj et al each reported a sensitization rate of 0.3% and 0.5% in the single centre study carried out in Singapore and Thailand respectively.[13,14] We did not differ from our neighbouring countries. The low sensitization rate in our cohort could be due to the lower exposure rate to class B corticosteroids.

Conclusion

The overall detection rate of contact sensitization increased by 3.4% with the addition of 1,3-Diphenylguanidine, N-Cyclohexyl-N-Phenyl-4-Phenylenediamine, N-Cyclohexylthiophthalimide and *Ylang ylang oil*. The addition of 1,3-Diphenylguanidine rubber in the baseline series improved the detection of rubber sensitization by 49.2% while the addition of *Ylang ylang oil* increased fragrance sensitization detection rate by 6.5%. A multicentre study with a larger local population is very much needed to support the inclusion of these allergens in the local screening baseline patch test series. Apart from that, more studies are needed to identify other locally relevant allergens to improve the detection rate of contact allergy in our population.

Conflict of Interest Declaration

The authors have no conflict of interest to declare.

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