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RESEARCH ARTICLE

DETECTION OF ALLERGENS FROM MITES BY SEMI QUANTITATIVE MEASUREMENT OF GUANINE FROM PATIALA, PUNJAB (INDIA).

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Abstract

176 infested dust samples were subjected to Acarex test and the corresponding risk evaluations have been analysed. 40 samples i.e. 22.7% of all the samples were under no Risk (step 0). 86 samples i.e. 48.8% of all the samples were under 'potential risk' (step 0.5, 1 and 1.5). 50 samples i.e. 28.4% of all the samples were under 'risk and high risk' (step 2, 2.5 and 3). It has been observed that 5.6% of the samples were under high risk therefore the homes from where these samples were collected shows great potential for allergic disorder.

Introduction:-

The development of allergies (allergic asthma, rhinitis, eczema) has been related with the exposure of human to both house dust and house dust mites. (Custovic et al., 1996; Nelson and Fernandez- Caldas, 1999). Mites that commonly subsist in human dwelling and provide a major source of multiple potent allergens are referred to as house dust mites. House dust mites from the family Pyroglyphidae (Acari: Acaridida) are recognised as the most important risk factors causing allergies in indoor environments (Fain et al., 1990; Arlian, 1991; Boulet et al., 1997; Korsgaard, 1998; Chew et al., 1999; Chowdhury and Chatterjee , 1999). Healthwise, it is important to know if the mite fauna has an undesirable effect on people. The most important of these, because of their cosmopolitan occurrence and abundance in homes, are *Dermatophagoides farinae*, *D. pteronyssinus* along with certain species of storage mites. Three species of house dust mites, *Dermatophagoides farinae*, *D. pteronyssinus* and *Euroglyphus maynei* are reported to be the major source of allergens in house dust (Arlian, 1991; Colloff ,1991; Arlian et al., 1993; Pauli et al., 1993; Robinson et al., 1997). More recently, the allergenic role of other species such as *E. maynei*, *B. tropicalis*, *L. destructor*, *G. domesticus*, *A. ovatus*, *T. putrescentiae*, *A. siro*, and *Chortoglyphus arcuatus* has been demonstrated (Fernandez- Caldas, 1999). The allergens produced by house dust mites are recognised with varying frequency and intensity by the sera of atopic patients. These allergens have been characterised and grouped according to their physicochemical similarities. These groups are:

Group 1 allergens of *Dermatophagoides*, *Der p* 1, *Der m* 1 and *Der f* 1, have a 24 kDa molecular weight and 80% chemical homology. The Eur m 1 allergen of E. maynei also belong to this group and show 80-90% recognition frequency by allergic patients.
Group 2 allergens are *Tyr p* 2, *Lep d* 2 and *Eur m* 2 from *T. Putrescentiae*, *L. Destructor* and *E. maynei* have a 14 kDa proteins and about 80% recognition frequency.
Group 3 allergens are *Der p* 3, *Der f* 3 and *Eur m* 3 with molecular weight of previous two are 24.9 and 30 kDa. These show 50% recognition frequency.
Group 4 allergens are *Der p* 4 and *Der f* 4 has 56 and 63 kDa molecular weight and 24 and 46% allergic frequency.
Group 5 have *Der p* 5 allergen with 14-15 kDa proteins and below 20% allergic frequency. *Blo t* 5 has 43% homology to *Der p* 5 and 45-60% recognition frequency.
Group 6 have *Der p* 6 and *Der f* 6 allergens with 25 kDa proteins and 40-60% recognition frequency.

Group 7 allergens are *Der p* 7 and *Der f* 7 22.1 kDa proteins and 86% homology.
Group 8 allergen is *Der p* 8 with 25-26 kDa molecular weight and 40% recognition frequency.
Group 9 allergen is *Der p* 9 with 24-29 kDa proteins and 80% homology.
Group 10 allergens are *Der p* 10 and *Der f* 10 has 33-37 kDa proteins and 76% homology.
Group 11 have *Der f* 11 allergen with 98 kDa molecular weight.
Group 12 and 13 allergens are *Blo t* 12 and *Blo t* 13 with 14 and 14.8 kDa proteins and 10% recognition frequency (Fernandez- Caldas, 1999).

The mites produce excreta which are released on every place where mites are living especially in textiles. After some months the textiles contain a mixture composed of dust, dirt and mite excreta. The mite excreta produced is about 200 fold the body weight of a mite (Van Bronswijk, 1981; Bischoff, 1989; Van Bronswijk et al., 1990). Recent studies show that the enzymatically active allergens are found in mite excreta. Those excreta allergens include a cysteine proteinase, several serine proteinases and glutathione-S-transferase. These proteinases remain active in mite excreta and have a direct potential to penetrate epithelial barriers. They can causes lesions in lungs when inhaled by sensitised human beings, the lung damaged by these enzymes are vulnerable to a wide range of other triggers such as rhinoviruses, bacteria or outdoor allergens (Solarz, 2001).

The excreta contain not only the mite allergens Der P1 or Der F1 but further on guanine as another important product of the mite metabolism. The guanine quantity is about 1000 fold the quantity of allergens and enables a visible chemical reaction to a guanine azo-dye (Bischoff and Schirmacher, 1984; Bischoff, 1989). All mites produce guanine during their life as a part of their metabolism, while the most commonly known major allergens like Der P1 or Der F1 are only produced by house dust mites of the species *Dermatophagoides*. This means that single allergens only depend on single mite species while guanine depend on all mite species present in a house or in a textile object. Thus guanine is a suitable marker for the risk evaluation related to persons sensitized against mite allergens (Pauli et al. 1988). It can be used for detection of allergens in the dust during onsite sampling.

Materials and methods:-

Detection of allergens from house dust:-

The fine dust sample of 2 gm was taken from the collected samples onto a watch glass of diameter about 6 cm, and 10 drops of extraction solution (methanol/water mixture in the weight ratio 90: 10, in which 4% by weight of NaOH are dissolved) and 10 drops of saturated aqueous diazosulphanilic acid solution were added. After briefly mixing, one side of a strip of white filter paper of defined size (0.5 cm) is pressed against the pasty mass. When there is mite infestation, a brick-red coloration emerges, and this is advantageously assessed via the unsoiled side of the paper (Warner and Mertz, 1989).

Depending upon the colour intensity the following parameters were made. Step 0 belongs to no risk, step 0.5 belongs to no risk (potential risk), step 1 belongs to potential risk, step 1.5 belongs to potential risk (risk), step 2 belongs to risk, step 2.5 belongs to risk (high risk) and step 3 belongs to high risk.

Observation:-

- 176 infested dust samples were subjected to Acarex test and the corresponding risk evaluations have been analyzed.
- 40 samples i.e. 22.7% of all the samples were under no Risk (step 0).
- 86 samples i.e. 48.8% of all the samples were under ‘potential risk’ (step 0.5, 1 and 1.5).
- 50 samples i.e. 28.4% of all the samples were under ‘risk and high risk’ (step 2, 2.5 and 3).

It has been observed that 5.6% of the samples were under high risk therefore the homes from where these samples were collected shows great potential for allergic disorder.

Table:- Risk evaluation of allergens from house dust using Acarex Test.

Step	Number	%age	Risk evaluation
0	40	22.7	No risk
0.5	20	11.3	No risk (potential risk)
1	52	29.5	Potential risk
1.5	14	7.9	Potential risk (risk)
2	25	14.2	Risk
2.5	15	8.5	Risk (high risk)
3	10	5.6	High risk

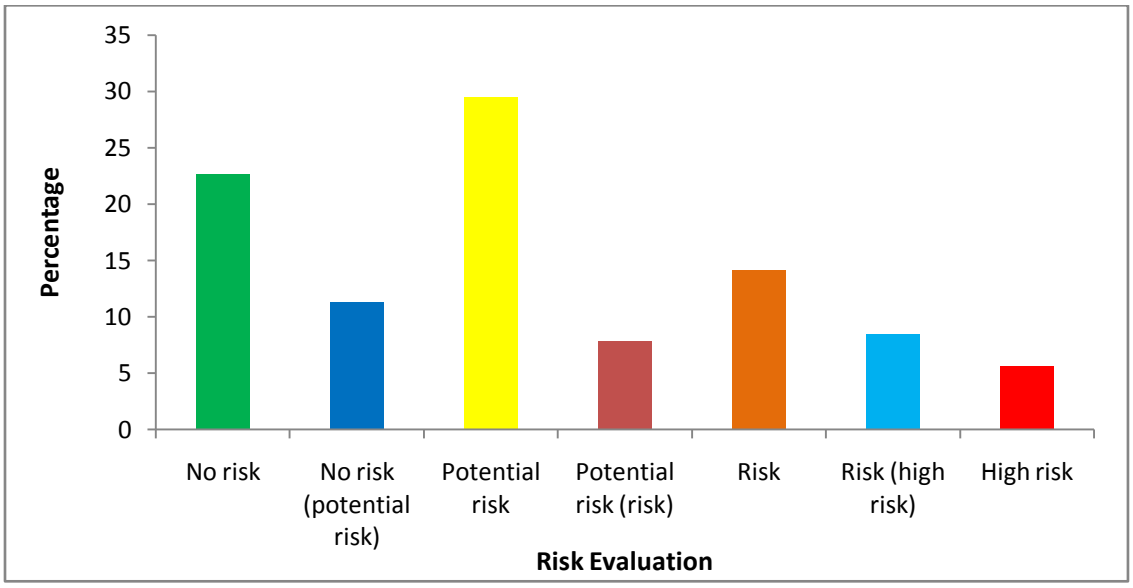

Fig.5:- Risk evaluation from allergens of house dust.

Discussion:-

Guanine is an important substance produced in the metabolism of house dust mites. The guanine quantity is about 1000 fold the quantity of allergens and enables a visible chemical reaction to a guanine azo-dye (Bischoff et al., 1984, 1989). According to Pauli et al. (1988) all mites produce guanine during their life as a part of their metabolism, while the most commonly known major allergens like *Der p* 1 or *Der f* 1 are only produced by house dust mites of the species *Dermatophagoides*. This means that single allergens only depend on single mite species while guanine depend on all mite species present in a house or in a textile object. Thus guanine is a suitable marker for the risk evaluation related to persons sensitized against mite allergens. It can be used for detection of allergens in the dust during onsite sampling. The development of a quick color reaction for guanine in house dust made possible to screen many homes for mite products of allergenic consequence. The azo dye methods are specific enough to be useful for the development of a sanitary standard. Comparing the guanine quantification results with mite counts and determinations of the mite antigen P1 (*Der p* 1) leads to the conclusion that the provisional standard for mattress dust of 0.6mg guanine/g dust can be extended to dust from floor coverings and padded furniture. During present investigations all the samples that gave azo dye reaction were found positive for mite species. Guanine is suitable as a marker for the allergen content of dust samples obtained from the inside of houses. Method for guanine detection is based on complete extraction of guanine out of the dust samples and the following guanine assessment. During present investigations in 6% samples, very high guanine contents have been detected. Therefore we can use guanine detection test (acarex test) as a preliminary test for detection of allergen content and concentration of mites in dust.

This study showed a diverse assemblage of house dust mite species occurring in human dwellings. Summarizing the present results reveals the occurrence of allergenic mites in close association with human and this undoubtedly can contribute to the incidence of respiratory problems. The relatively high densities of allergenic mites are a cause of concern. The high abundance and frequency of *Blomia tropicalis* in dust samples indicate that species plays an

allergenic role as important as *Dermatophagoides farinae* in asthma and allergic rhinitis. Some of these allergenic mites show distinct monthly distribution patterns which can be used to forecast their peak abundance and in the process provide an early warning to allergic individuals.

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