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Comparison and analysis of IgE and blood histamine levels: its correlation during allergic sensitization in atopic and non-atopic population

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Abstract

Independent and inter-dependent troupe of histamine- an inflammatory immune mediator together with Immunoglobulin E (IgE) and mast cells shoulder the responsibility of clinical allergic sensitization in atopic population which ends-up in the severe anaphylactic and acute allergic reactions. Histamine with their receptors (H1R-H4R) plays a significant managerial role in several allergic diseases. In this study an emphatic effort has been employed to screen, evaluate and compare to understand the serum and plasma histamine levels in atopic and non-atopic populations and in turn correlated with total IgE levels to understand interrelation of IgE with histamine content during allergic reactions. The atopic and non-atopic subjects were selected based on subjective case histories and were analysed for blood Eosinophilic count, Total IgE levels and plasma and serum histamine levels. Leukocyte isolation, rat peritoneal exudate mast cells isolation and histamine release quantification and their HPLC screening, finally correlating the total IgE with Histamine released were assayed by comparing the values obtained with atopic, non-atopic samples and with known standard values of histamines, leukocytes, eosinophil's of Normal healthy people.

Key words : IgE, Blood histamine, Allergic sensitization, HPLC screering.

Ligation of Immunoglobulin E molecules on cell surface, was occurred by an allergen or bi-valet antibody against IgE is a requisite event for IgE-induced basophil or mast cell degranulation processes and this interactions induce release of histamine and other including inflammatory mediators[29]. With respect to hypersensitivity (immediate) and hypersensitivity related diseases, mast cells are the significant effector and regulatory auditing cells[7,14]. Cross-linking of IgE molecules with greater affinity bound to FcεRI of mast cells with polyvalent antigens trigger the activation of mast cells by elevating the granulation of FcεRI[39]. This kind of FcεRI -reliant activation upshots the degranulation of cytoplasmic granules namely vasoactive amines-Histamines, proteoglycans and neutral proteases. De-novo coordinated synthesis of lipid mediators (pro-inflammatory), production and secretion of cytokines, chemokines. Both chemical as well as lipid mediators furnish to the expansion of allergy and different forms of inflammation[6]. Asthama, atopic dermatitis, rhinitis, pruritus and rarely anaphylaxis are some of the allergic diseases occurred by the complex interaction of various inflammatory cells along with mast cells, basophils, dendritic cells, eosinophills and neutrophils giving rice to numerous allergic stimuli[13]. Among these mediators, mast cell histamines greatly contributes in stimulating the progress of allergic-dependent inflammatory diseases by monitoring the maturation of leukocytes and their activation, addressing their migration towards target sites and bring about chronic inflammation[2,9]. Histamine (2-[4-imidazolyl] ethylamine) was identified as a mediator of anaphylactic and allergic reactions. Histamine belongs to the biogenic amines and is synthesized by the pyridoxal phosphate (vitamin B-6) containing L-histidine decarboxylase HDC[4]. From the amino acid histidine[15]. It is synthesized by mast cells, basophils, platelets, histaminergic neurons, and enterochromaffine cells, where it is stored intracellularly in vesicles and released on stimulation[16]. Histamine is a potent mediator of numerous biologic reactions[21]. Besides the well-known triggering of degranulation of mast cells by crosslinking of the FcεRI receptor by specific allergens[3]. Histamine exerts its effects by binding to its four receptors [histamine 1 receptor (H1R), H2R, H3R, and H4R] on target cells in various tissues[41]. It causes smooth muscle cell contraction, vasodilatation, increased vascular permeability and mucus secretion, tachycardia, alterations of blood pressure, and arrhythmias, and it stimulates gastric acid secretion and nociceptive nerve fibers[27,37]. Hyperhistimanic common symptoms occurred by binding with the readily available H1-receptor in both children and adult were consolated by the antihistamines H1R-antagonists namely levocabastine[11]. H2-receptor released by the enterochromaffin-like cells in circulating gastrin results in the excessive secretion of gastric acid and hence causes gastric ulcer which was consolated by antihistamines H2R-antagonists namely ranitidine[18]. H3-receptor stimulation results in the norepinephrine release suppression at presynaptic nerve endings and meantime secrets the nasal sub mucosal gland[36]. H4-receptor existed on basophils, neutrophils, mast cells and nasal nerve turbinates. Can also be found on colon, bone marrow, liver, spleen and lung. H4R induce chemokinesis and chemotaxis of mast cells and eosinophills[22]. In addition, histamine has been known to play various roles in neurotransmission, immuno-

modulation, hematopoiesis, wound healing, day-night rhythm, and the regulation of histamine- and polyamine-induced cell proliferation and angiogenesis in tumor models[1,26]. Basal plasma histamine concentrations of 0.3 to 1.0 ng/mL are considered normal. Exceeding the individual histamine tolerance gives rise to concentration-dependent histamine mediated symptoms[32]. Even healthy persons may develop severe headache or flushing due to ingestion of massive amounts of histamine as is known from studies of scromboid poisoning[5,10]. It has been shown that inhibition of DAO followed by oral histamine administration may induce severe and even life-threatening reactions, such as hypotension, bronchospasm, or shock[20]. Recurrent anaphylactic reactions have been reported in patients with hyperhistaminemia[25]. It was observed that symptoms occur even after the ingestion of the small amounts of histamine that are well tolerated by healthy persons[19]. Symptoms can be manifest via the above mentioned actions of histamine in multiple organs, such as the gastrointestinum, lung, skin, cardiovascular system, and brain, according to the expression of histamine receptors[12,17]. Typical symptoms of histamine intolerance include gastrointestinal disorders, sneezing, rhinorrhea and congestion of the nose, headache, dysmenorrhea, hypotonia, arrhythmias, urticaria, pruritus, flushing, and asthma[23,34]. Present research study of investigation made an attempt to understand and to study the histamine levels in atopic and non-atopic blood sample and also correlate with the serum IgE levels, to understand the relationship of IgE with histamine levels in assessing the severity of allergic reactions.

Materials :

This study was undertaken after clearance by the Institutional Human Ethics Committee; informed consent was obtained from all atopic and non-atopic subjects in the age range of 15-60 years (for subjects below 18 years of age, consent were obtained from their parents or legal guardian). Compound 48/80, concanavalin (con A), murine anti-human IgE (monoclonal)-alkaline phosphatase (AP) conjugate were products of Sigma-Aldrich Co., St. Louis, MO, USA. Lysozyme, ovalbumin (OVA), and bovine serum albumin (BSA) were purchased from Bangalore Genei, Bangalore, India. Dextran T 700, *o*-pthalaldehyde (OPT) were products of Hi-Media Laboratories, Mumbai, India. Flat-bottom 96-well microtiter plates (MICROLON) were bought from Greiner Bio-One GmbH, Frickenhausen, Germany. All other chemicals/reagents used in this study were of analytical grade. Compound 48/80, chitosan, pepsin, avidin, concanavalin (con A), *Lycopersicon esculentum* agglutinin (LEA; tomato lectin), sheep anti-mouse IgG (whole molecule)-AP conjugate, and murine anti-human IgE (monoclonal)-alkaline phosphatase (AP) conjugate were products of Sigma-Aldrich Co., St. Louis, MO, USA. Flat-bottom 96-well microtiter plates (MICROLON) were bought from Greiner Bio-One GmbH, Frickenhausen, Germany. All other chemicals/reagents used in this study were of analytical grade.

Identification of atopic and non-atopic subjects :

These subjects were identified based on case history (atopic subjects are chosen at random who had symptoms of at least one allergic condition such as allergic rhinitis, atopic dermatitis, asthma, food allergy, and allergic

conjunctivitis) of the subjects and skin prick tests (SPT) of certain commercial pollen and house dust mite (HDM) extracts. Most of the subjects examined in this study were from the rural areas. SPT was performed using various commercially available allergen extracts such as grass mix 1, grass mix 2, (pollen extracts from various kinds of grasses which contain mixture of allergens), weed mix (pollen extracts from different weeds) and house dust mite (HDM) (which contain the feces of the dust mite) that is allergic to 20% of the total atopic subjects. The serum samples of the atopic subjects were collected from the allergy camp and were used for the study. The serum obtained from the healthy volunteers without any symptoms allergy were used as no-atopic controls.

Eosinophil count, serum total IgE levels, serum and plasma histamine levels :

Absolute eosinophil counts in the blood of atopic and non-atopic subjects :

It is important to carry out a direct total or absolute eosinophil count to get an accurate value of total eosinophil count[28,31] for determination of absolute eosinophil count, 380 μL of diluting fluid was taken. [The diluting fluid used should stain the eosinophil's and lyse the red blood cells; commonly used diluting fluid was Hingleman's solution that contains yellow eosin (0.5 g), 95% phenol (0.5 mL), 40% formalin (0.5 mL) and distilled water 99.0 mL]. To this, 20 μL of blood was added and mixed. The sample was then kept at 25°C for 10-15 min before charging to the hemocytometer counting chambers. Alternatively, the counting chamber may be filled immediately to prevent clumping of eosinophils and kept in a moist chamber for 5 min in a Petridish for cells to settle and lyse RBC. The eosinophils were counted in all the 9 squares with low power (10 X) eyepiece. The following formula is used for the calculation:

$$\text{Total number of Eosinophils} = \frac{(\text{No. of cells counted}) \times (\text{dilution factor}) \times (\text{depth factor})}{\text{No. of areas counted}}$$

$$= \frac{z^* \times 20 \times 10}{9} = \text{eosinophils / } \mu\text{L.}$$

(* corresponds to the number of cells counted)

For a reasonably accurate count, at least 100 cells must be counted and lower counts are obtained if there is no proper filling of the counting chambers. The normal value of eosinophils is 40-400 cells / μL in healthy individuals.

Serum separation from the subjects' blood:

Ten milliliters of venous blood was drawn from the subject into a clean sterilized 15 mL polystyrene tube. The tube was plugged with cotton and allowed to stand at room temperature for 1 h. After clotting, it was kept at 4°C for 2 h and then centrifuged at 750 x g at room temperature for 10 min. The separated serum was aspirated and stored in aliquots at -20°C until analysis.

Determination of total IgE levels in serum:

Total IgE levels in atopic and non-atopic subjects was detected by enzyme-linked immunosorbant assay (ELISA) using anti-human IgE antibodies[8] (murine IgG2a, κ; hybridoma cell line ATCC HB-121, designation E5BB3IIA2, obtained from National Centre for Cell Science (NCCS), Ganeshkhind, Pune,

India) which was purified from hybridoma cell culture supernatant on protein A-agarose column affinity chromatography. For the total IgE determination, known concentration of monoclonal murine IgG2a (kappa) that reacts with human IgE (ATCC, 1985) was coated (100 µL/well) on microtiter plate coating buffer (0.1 M carbonate-bicarbonate buffer, pH 9.6) at 4ºC overnight. After washing using phosphate-buffered saline (PBS) containing 0.05% Tween-20, abbreviated as PBS-T, blocking reagent (3% gelatin prepared in PBS) was added and plate was incubated at 37 ºC for 1 h. Then serum samples (100 µL/well) from non-atopic or atopic subjects, appropriately diluted (1:3) with diluent buffer (PBS containing 1% BSA and 0.5% Tween-20) were added to each well. The plate was then incubated in a humidified chamber at 37°C for 2 h. After incubation, 100 µL of secondary antibody [murine anti-human IgE antibody conjugated with horseradish peroxidase at a dilution of 1:2000 in diluting buffer (PBS containing 1% BSA, 0.5% gelatin and 0.05% Tween-20)] was added and the plate was incubated at 37°C for 2 h in humidified chamber. Finally, 100 µL of freshly prepared horseradish peroxidase substrate solution (0.5 mg/mL *o*-phenylene-diamine and 0.006% H_2O_2 in 0.2 M potassium phosphate buffer, pH 7.0) was added and allowed to stand at 25 °C in the dark for 20 min. The enzyme reaction was terminated by the addition of 100 µL of 2 N HCl, and the absorbance read within 15 min at 492 nm in a ELISA reader. Values are expressed as ELISA units with one ELISA unit defined as one absorbance unit at 492 nm.

Histamine quantitation assay :

Histamine was quantitated by fluorometric assay using *o*-pthalaldehyde OPT as a fluorescent reagent. Histamine stock (10 mg/ mL) was prepared by weighing 16.2 mg of histamine dihydrochloride, which corresponds to 10 mg of histamine base in water. The histamine standards were prepared in the concentration range of 0-50 ng in the volume range 0-1000 µL. The histamine content in the serum or plasma was quantitated following an extraction method[24,41]. Briefly, 100 µL of the serum or plasma (after treatment with 10% TCA for protein precipitation) was diluted to 1 mL using PBS. The supernatant containing histamine and other low mol. wt. mediators were extracted by manual extraction method using *n*-butanol-heptane system into 0.1 M HCl. The tubes containing histamine were made up to 1 mL using 0.1 N HCl and transferred to an ice bath and to each tube, 0.3 mL of 1 N NaOH was added and immediately 75 µL of 0.2% OPT solution was added and mixed. The reaction was allowed to proceed for 40 min in an ice bath. Then, 0.15 mL of 2 M H_3PO_4 was added, mixed and kept at room temperature for 15 min. The samples were read in a spectrofluoropho-tometer (Ex λ: 360 nm; Em λ: 450 nm).

Isolation of leukocytes containing basophils:

Leukocytes (buffy coat containing basophils) were isolated from 10 mL of heparinized venous blood as described[15] using 6% dextran T 700 gradient. The leukocyte layer was washed 4-5 times with isotonic PBS and resuspended in Tris-CAM buffer. The isolated leukocytes were counted using crystal violet; cell viability, as determined by Trypan blue dye exclusion, was 95%.

Histamine release (HR) assay :

After cell count and viability test, nearly 1.5 - 2 x 10^6 cells /mL were taken for the histamine release assay. This assay consists of three steps[33].

Step 1: Incubation of cells with lectins to release histamine :

For histamine release, different concentrations of lectin were added (in a volume of 0.25 mL) to polystyrene tubes containing 0.5 mL TrisCAM buffer in an ice bath followed by cells 0.25 mL (from buffy coat and peritoneal exudates) to all tubes[29,38]. The tubes were transferred to a 37°C water bath and incubated for 45 min. Reaction stopped after transferring the tubes to an ice bath. After centrifuging the tubes at 1600 rpm at 4°C for 20 min, the supernatant was poured off and assayed for histamine content (*test release*). In each set of experiment, 10% perchloric acid was added to two tubes (without lectin samples) to a final concentration of 3%; two additional tubes (without lectin samples) were boiled at 100°C for 10 min; both these manipulations are used to obtain the total histamine content of cells (*complete release)*. Blank tubes containing only cells and buffer are used as controls for obtaining the basal level of histamine release (*spontaneous release)*. The histamine release in these tubes was always less than 10% compared to complete release. All the experiments were done in duplicate; there was <2% variation between duplicate tubes.

Step 2: Manual extraction of released histamine :

The supernatants after centrifugation were poured into separate polystyrene tubes. One mL of this was added to a tube containing 0.3 g NaCl and 1.25 mL butanol. After addition of 0.1 mL of 3 N NaOH, the tubes were shaken and centrifuged at 600 × g at 4°C for 15 min. One ml of butanol (top) layer was removed with a pipette and transferred to another tube containing 0.6 ml of 0.12 N HCl and 1.9 mL of n-heptane. Tubes were shook for 1 min by inversion, and allowed to stand for 5 min for clear separation of two phases. Next 0.5 mL of 0.12 N HCl layer (bottom layer) containing histamine was carefully aspirated using a fine tip and transferred to another tube for histamine assay[30]. Care should be taken while removing the HCl bottom layer since butanol may interfere in the histamine assay.

Step 3: Histamine release assay by HPLC method :

Histamine release assay was carried out as explained above for the fluorometric method, then the released histamine was condensed with *O*-pthalaldehyde to form a fluorescent condensation product and assayed by HPLC method. Each test sample along with standard were prepared and assayed by HPLC using C_{18} column using a mobile phase containing solvents A and B at a ratio of 5:95. Solvent A is methanol and solvent B is a mixture of 0.2 M NaCl and methanol at 45:55. About 20 µL of each sample was injected and elution was carried out at a flow rate of 0.8 mL/min and monitored using a fluorescent detector at Ex_λ of 355 nm and Em_λ of 450 nm.

Step 4: Quantitation of histamine by fluorometric method :

The tubes containing 0.5 mL of the

extracted histamine was added with 0.5 mL of 0.12 N HCl, and was transferred to an ice bath[35]. To that, 0.2 mL of 1 N NaOH was added, and immediately 0.050 mL of 0.2% *o*-pthalaldehyde (OPT) solution was added and mixed. The reaction was allowed to proceed for 40 min. After incubation, 50 µL of 2 M H_3PO_4 was added to stop the condensation reaction and mixed. Then the tubes were kept at room temperature for 15 min. The fluorescence intensity of the solutions was measured using an excitation wavelength of 360 nm and emission wavelength of 450 nm using a spectrofluorometer.

Formulae for the calculation of percent histamine release and percent inhibition :

$$\text{Histamine release (A \%)} = \frac{(Pt - Ps)}{(Pc - Ps)} \times 100$$

Where, Pt refers to histamine in the test release
Ps refers to histamine in the spontaneous release
Pc refers to histamine in complete release
A% is percent histamine release

$$\text{Inhibition (I \%)} = \frac{1 - (S - B)}{(C - B)} \times 100$$

Where, S is percent histamine obtained from test release
C is percent histamine obtained from control release
B is percent histamine obtained from spontaneous release
I % is percent of inhibition for histamine release.

Histamine release assay by HPLC method :

Histamine release assay was carried out as explained above for the fluorometric method, then the released histamine was condensed with *O*-pthalaldehyde to form a fluorescent condensation product and assayed by HPLC method. Each test sample along with standard were prepared and assayed by HPLC[41] using C_{18} column using a mobile phase containing solvents A and B at a ratio of 5:95. Solvent A is methanol and solvent B is a mixture of 0.2 M NaCl and methanol at 45:55. About 20 µL of each sample was injected and elution was carried out at a flow rate of 0.8 mL/min and monitored using a fluorescent detector at Ex_λ of 355 nm and Em_λ of 450 nm.

Statistical analysis :

Each datum represents the mean and standard error of the mean (SEM) of the different experiments under identical conditions. Student's *t*-test was used to make a statistical comparison between the groups.

Selection of atopic and non-atopic subjects for the study :

Atopic and non-atopic subjects were selected based on detailed case history and clinical symptoms for house dust mite/pollen allergy. Among these groups, the atopic or non-atopic status was confirmed in a representative number of subjects (n = 12 for atopics, and n = 10 for non-atopics) based on the eosinophil count, serum total IgE and plasma/serum histamine levels.

Absolute eosinophil counts in non-atopic and atopic subjects :

Based on the eosinophil counts and on the incidence of allergic reactions and symptoms, the subjects were classified as non-atopic and atopic subjects. The eosinophil

Table-1. Absolute eosinophil counts in atopic and non-atopic subjects

Subjects	Eosinophil counts/μL*	
	Mean ± SEM	Range
Non-atopic (n=10)	302 ± 11	240 – 350
Atopic (n=12)	776 ± 18	680 – 860

*Reference normal value for eosinophil counts = 40–400 cells/μL [29]; $p \leq 0.001$ ($t = 38.2$)

counts were expressed as mean value obtained in each category with standard error of mean. For non-atopics, the value was found to be 302 ±11 with a range of 240 – 350 cells/μL of blood. In atopic population, the eosinophilic counts were found to be 776 ± 18 with a range of 680 - 860 cells/μL of blood. The eosinophil counts were increased by ~2.6-fold over the mean value for non-atopic subjects.

Total IgE levels in normal and allergic sera:

The total (circulating) IgE levels in the sera of non-atopic and atopic subjects were determined using ELISA. The total IgE exhibited a wide range among atopic subjects (177.3–330.1 IU/mL) as compared to non-atopics range (34.7–43.9 IU/mL) were mentioned in table-2.

Table-2. Total IgE levels in atopic and non-atopic subjects

Subjects	Total IgE levels		
	ELISA units (492 nm)	Mean ± SEM	Total IgE* in IU/mL (range)
Non-atopic (n=10)	0.248 – 0.314	0.267 ± 0.008	34.7 – 43.9
Atopic (n=12)	0.844 – 1.558	1.205 ± 0.120	177.3 – 330.1

*Reference normal value for serum total IgE = <120 IU/mL [8] , $p \leq 0.001$ ($t = 13.40$)

The normal range for total IgE is <120 IU/mL. The serum total IgE was found to be significantly higher in atopic subjects, and represents approximately a 5-7 fold increase over the value for non-atopic subjects.

Serum and plasma histamine levels in allergic and normal subjects :

Histamine standard curve was obtained in the range of 0 - 100 ng histamine base (1.66 mg of histamine.2HCl is equivalent to 1 mg histamine base) in a volume range of 0 to 1 mL. A linear response was obtained with a high correlation having correlation coefficient (R^2) value of 0.997. Were shown in figure 1.

Serum and plasma histamine levels were quantitated in atopic and non-atopic subjects. The serum and plasma histamine levels were found to be significantly higher in atopic subjects (~6 to 8-fold) as compared to the mean value for non-atopics. The plasma histamine level is 5 to 10-fold lower compared to serum histamine levels in each group. The normal reference value for serum histamine was 5-27 ng/mL whereas for plasma histamine was 0.5 to 2 ng/mL in non-atopic healthy subjects were mentioned in table-3.

Table-3. Histamine levels in the serum and plasma of atopic and non-atopic subjects

Subject	Histamine content in serum (ng/mL)*		Histamine content in plasma (ng/mL)#	
	Mean ± SEM	Range	Mean ± SEM	Range
Non-atopic (n=10)	28.2 ± 3.6	21 – 32	1.5 ± 1.2	0.6 - 3.0
Atopic (n=12)	184.2 ± 10.1	155 – 215	11.6 ± 1.0	9.6 – 14.8

*Value for non-atopic subjects is 5–27 ng/mL; $p \leq 0.001$ ($t = 15.74$). #Value for non-atopic subjects is 0.5 to 2 ng/mL; $p \leq 0.001$ ($t = 10.64$)[24].

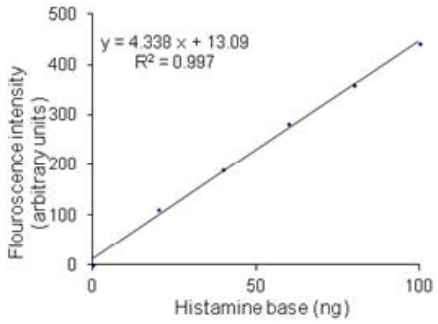

Fig. 1. Histamine standard curve, concentration range 0-20 ng. R2= 0.997

Selection of atopic and non-atopic subjects for the study :

Subjects classified as non-atopic never had a history of any allergic diathesis and had negative SPTs, normal eosinophil counts, and normal IgE levels. Subjects classified as atopic had one SPT positive for any of the inhalants with a clinical history and examination suggestive of allergic rhinitis, asthma or both. Evaluation of atopic cases, including those avoiding potato ingestion, was carried out by complete clinical case history in the form of questionnaire. The atopic condition exhibiting allergic rhinitis and asthma was confirmed by physical examination, clinical symptoms and spirometry. Based on the above criteria, the allergic status of the subjects was classified arbitrarily as non-atopic or atopic. Total IgE, and histamine levels are summarized in Table-4.

Table-4 Demography of the selection of non-atopic and atopic subjects for the study

Parameters	Subjects' status	
	Non-atopic	Atopic
Gender (M/F)	11/9	53/57
Serum total IgE (IU/mL)†	35-44	59-330
Plasma histamine (ng/mL)#	1.2-2.4	8.6-13.4
Serum histamine (ng/mL)‡	21-32	110-215

*Allergen extracts include various food, and commercial pollen extracts; wheal/flare diameter (range): histamine base, 5-6/20-25 mm; glycerinated PBS, 0-1/0 mm; HDM, house dust mite; SPT grading: 1+, 3-4/5-10 mm; 2+, 4-5/10-20 mm; 3+, >5/20-30 mm

†Reference normal value for serum total IgE = <120 IU/mL

#Reference normal value for plasma histamine = 0.5-2 ng/mL

‡Reported range for serum histamine (normal subjects) = 5–27 ng/mL

Histamine release (HR) from atopic subject as determined by HPLC assay :

The pattern of histamine release measured by HPLC assay agreed well with the results from the manual extraction of histamine by fluorometric method. STA was used in the concentration range of 1-3 µg/mL. The HPLC pattern obtained at different amounts is shown in Fig. 2. The maximal release was found at 2 µg of non-specific lectin (LEA) where the peak at 7 min (representing histamine peak) shows a maximum area. The peak area of the Pc was considered as 100% release, and the Ps as standard blank. The percent histamine release was calculated and found to be in good agreement with the histamine release measured by the fluorometric method.

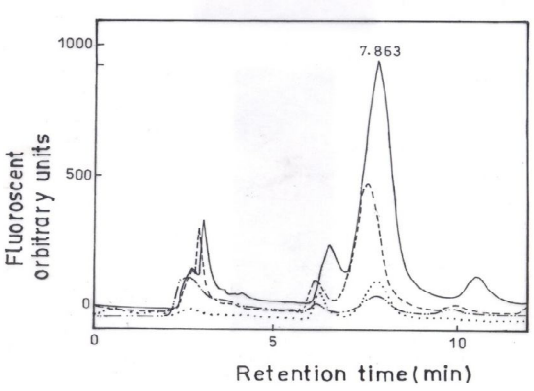

Fig. 2. Histamine release from the leukocytes of atopic subject assayed by HPLC method. The histamine was detected with a retention time of 7.863 min. (_._ reagent blank, …. Ps, Pc, ---- LEA at 2 µg)

Correlation of HR with total IgE :

Ten subjects in the non-atopic group and 20 subjects in the atopic group were analyzed for HR and total IgE levels. The results are shown in Figure 3. The percent HR was found to have a strong correlation with the serum total IgE levels (R^2 = 0.923, n = 30). The mean value of percent HR as well as the range of serum total IgE values for both non-atopic and atopic (including its sub-groups) groups are shown in Table-3. The correlation between Total IgE levels in the atopic subjects and the percent histamine release from the leukocytes isolated from the same atopic subjects, and the skin prick test data, with clinical history of subjects suggests the non-specific activation of basophils/mastcell through cell bound IgE by lectin (LEA) depends on total IgE levels.

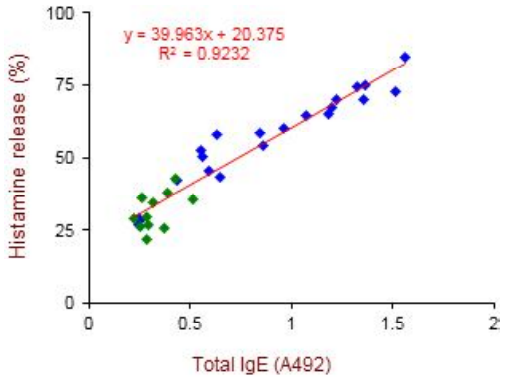

Fig. 3. Correlation of leukocyte histamine release to serum total IgE (ELISA units at A_{492}), n = 30. (◊), non-atopic subjects (n = 10); (♦), atopic subjects (n = 20).

Histamine is an authoritative mediator of immediate hypersensitivity reaction which is stored to a large extent in mast cell and basophils. H1R and H2R are the main cell surface receptors of histamine and interaction with this receptors histamine operates pharmacologic effects which introduce the changes in vascular-permeability, cyclic

nucleotide parameters, smooth muscle contraction, chemokines, gastrointestinal secretion eosinophil, neutrophil and chemotaxis. Antihistamine medications related to H1and H2 receptors have been anciently employed to interrupt these allergic responses and also certified clinically beneficial in the treatment of certain categories like urticaria, peptic ulcer and rhinoconjuctivitis. Numerous biochemical assays have been advanced to estimate histamine release in blood, urine and tissue from mast cells and basophils. In this study a Clear cut brief summary of histamine release screening was done by careful selection of Non-atopic subjects and atopic subjects with case history of allergy along with minimum of 3mm diameter greater than that of negative control in skin prick test. Coming to the detailed step by step screening involves initially, with definite eosinophil counts of non-atopic subjects were increased by ~2.6-fold over the mean value compared with standard referral normal value. Similarly serum Total IgE was found approximately 5-7 fold increase over the value for non-atopic subjects compared with standard. Sera and plasma histamine levels were found approximately 6 to 8-fold increase over the value for non-atopic subjects compared with standard. 1+,2+ and 3+ SPT grading was obtained with atopic subjects. Finally 100% Leukocyte histamine release was captured by HPLC peak screening at 7min and correlation of leukocyte histamine release with total IgE indicate the non-specific activation of basophil/mast cells.

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Conflict of Interest: The authors declare that there are no conflicts of interest.

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