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Original Research Article

A Novel Method for Treating Bronchial Asthma with Newly Designed Histaglobulin Therapy

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Abstract: Asthma, a chronic inflammatory disease of the airways is considered as an incurable disease and extensive research is going on in developing novel treatment. The objective of the study is to establish that the antihistamine antibody has low serum histamine binding capacity (SHBC) in allergic patients as compared to normal persons and that it can be raised with our novel method of Histaglobulin treatment thereby effecting a 'cure' in bronchial asthma. About 67 normal and 135 allergic persons, were identified based on serum level of total immunoglobulin E (IgE) estimated by ELISA. Antihistamine antibody was separated from serum by affinity chromatography. Presence of antihistamine antibody in the elute was confirmed by SDS-PAGE test. The antihistamine antibody was IgG, confirmed by MALDI-TOF analysis. The SHBC of the antihistamine antibody was estimated based on standard curve, plotted with different concentrations of serum antihistamine antibody by histamine ELISA test. Total Immunoglobulin E and SHBC were estimated in 135 allergic patients. In normal and allergic persons, the total IgE level was <100 IU/ml and 200 IU/ml respectively. The average SHBC of normal persons was 116 µg/ml, which was 39% more as compared to allergic patients whose average SHBC before treatment was 83.6 µg/ml. 135 allergic patients were first subjected to antihistamine, mast cell-membrane stabilizers, anti-inflammatory and bronchodilator drugs followed by histaglobulin injection. The SHBC level was raised in 122 (90.4%) patients to an average of 111.6 µg/ml. Overall, 132 (97.8%) allergic patients responded well to our treatment and were free from allergic manifestation throughout the follow-up period of about 5 years. Hence, this novel treatment with histaglobulin which has scientific basis, effective and well tolerated can be adopted across the world for effecting a cure from bronchial asthma and allergic rhinitis.

Key words: Bronchial Asthma, Serum Histamine Binding Capacity, Histaglobulin, Histamine ELISA

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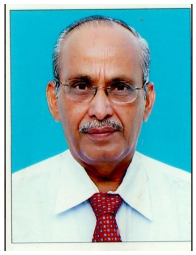

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1. INTRODUCTION

Asthma is defined as a chronic inflammatory disease of the airways in which, inflammation is the central event leading to the development of bronchial hyper reactivity and asthmatic symptoms.[1] Early reports on the prevalence of asthma and allergic diseases was provided by European Community Respiratory Health Survey (ECRHS 1996) and International Studies on Asthma and Allergies in Childhood (ISAAC 1998).[2,3] Globally, about 300 million and in India about 18-20 million people are suffering from bronchial asthma and globally about 1,80,000 people die every year[4]. Since the exact cause of allergic asthma is not known, various hypotheses have been put forth as causative factors for its occurrence of allergic asthma. Some of the key causative factors includes increasing exposure to aggressive factors such as airborne pollutants high salt intake, indoor allergens, drugs and vaccines[5-8] while microbial load and antioxidants seem to have a protective effect[9,10]. Overall, treatment for allergic asthma is still an unmet medical need, since effective cure for the disease is eluding. Currently available epidemiological evidence suggests that the role of allergen exposure and atopy in the development of asthma may have been over-emphasized. Hence, alternative paradigms based on other etiological mechanisms and risk factors need to be explored. The studies conducted by Ninan and Russell (1964-1989)[11], Rimpela et al., (1977-1991)[12] and Peat et al., (1991- 1993)[13] suggested that atopic diseases arise from a combination of genetic, environmental and immunological influences. In addition to genetic factors, various hypotheses on the potential cause for disease development have been proposed that include non-genetic factors such as, allergen, diet, hygiene, ante-natal and post-natal infection and intestinal microbial flora. Stannegard (1978)[14] reported a primary defect of lower number of lymphocytes in atopic children. Inhaled corticosteroids (ICS) are still used widely as one of the treatment options. ICS do not cure the disease but only alleviate the signs and symptoms[15]. Hence extensive research work is going on in developing novel treatment options such as counteracting Th2 cell activation by inducing the endogenous production by exogenous administration of antagonizing cytokines such as IL-12, or IL-10[16, 17], limiting Th2 cell development by antagonizing the cytokines produced by Th2 cells with anti-IL-4, anti-IL-5 antibodies[18], alleviating cytokine production by inhibiting p38 MAP kinase activity[19]. Other commonly used approaches include use of anti-IgE antibodies[20], selective PDE4 inhibitors[21] and airway remodeling through selective inhibition of matrix metalloproteinases (MMP2) and MMP9[22]. Most, if not all of these approaches focus on mechanisms involved in disease pathogenesis and also only offer a symptomatic relief and not a complete cure. Epidemiological studies conducted in different countries by various groups showed that the occurrence rate was more or less same in urban, rural and industrial area. Similar findings were reported by Alameldin (2012)[23] Gaur (2006)[24], Wilkie (1995)[25] and Xu (1993)[26] from studies conducted in Egypt, India, New Zealand and China, respectively, where prevalence of asthma was comparable between urban, rural and industrial areas. Epidemiological study of asthma in and around Pollachi, Tamilnadu, India, conducted by us earlier and a study conducted by Von Mutius (1994) established that the prevalence rate of asthma was about 10 to 15% only[27, 28]. People across various regions are exposed to the same air pollutants, but only 10 to 15% of the population develop allergic reaction, and remaining 85% to 90% of the people are not affected. Although, the vast majority of us are exposed to the same allergens and atmospheric pollutants, there is something inherent within our system that protects us from manifestation of allergic reactions. Therefore, the actual causative factor that leads to allergic reactions in an individual should probably be within the person. It was reported by Parrot et al., (1953)[29] that the serum of normal individuals possessed 20-30% more histamine binding capacity, as compared to allergic patients. This low level of histamine binding capacity in allergic patients appears to be the cause for allergic diseases like bronchial asthma, allergic rhinitis and other similar diseases. Based on this, we hypothesized that, bronchial asthma and allergic diseases can be cured if SHBC (Serum Histamine Binding Capacity) can be raised in allergic persons and devised a novel method of histaglobulin administration. In our earlier poster presentation (World Allergy Congress held on December, 2007)[30], we clearly showed that our modified method of histaglobulin treatment was effective in curing allergic bronchial asthma. In that study, conducted with 161 allergic patients, 152 patients (94.6%) were completely cured of all clinical symptoms by following our treatment method. In this present study, SHBC was estimated in normal as well as allergic persons to understand if it is inherently lower in allergic patients. Total IgE and SHBC were estimated in these allergic patients before and after treatment with histaglobulin to evaluate if there are changes in these parameter in patients who became free from allergic manifestations.

1.1 Objective

The objective of the study was to evaluate if SHBC is significantly different in allergic vs. normal individuals. Further, we also wanted to understand, if SHBC level is modulated in patients who became free from allergic manifestations after treatment with our newly devised histaglobulin treatment.

2. MATERIALS AND METHODS

Patients who visited Asthma Research and Treatment Center for treatment of bronchial asthma or other allergic diseases were selected based on inclusion criteria. Diagnosis of asthma was based on the GINA guidelines[31]

2.1 Inclusion criteria

2.1.1 For normal persons

- No history of atopic diseases such as allergic rhinitis, bronchial asthma, urticaria, atopic dermatitis and others.
- No family history of above mentioned atopic diseases
- Total Ig E level in the serum <100 IU/ml

2.1.2 For allergic patients

- Definite history of at least 3 previous attacks of wheezing
- Increased total Ig E level in the serum (>200 IU/ml) as estimated by ELISA
- Family history of atopic diseases

2.2 Exclusion criteria

The following cases were excluded

- Those with organic heart diseases
- Those with organic pulmonary diseases

- Those with secondary pathological changes such as fibrosis or bronchiectasis or emphysema

2.3 Collection of blood samples

Blood samples were collected after obtaining patients' written consent. Patients' blood pressure was checked and thorough medical examination was carried out. Using 5 ml sterile syringe, 3 ml of blood was collected by venipuncture under strict aseptic precautions. Blood from the syringe was transferred to sterile 10 ml test tube and centrifuged to separate serum. Serum thus obtained was used to carry out all the studies mentioned below.

2.4 Estimation of IgE and food allergens

Total IgE for normal and allergic individuals was estimated by ELISA as per the instructions from manufacturer (Omega Diagnostics, UK). Specific IgE level for food allergens were estimated by Euroline test kit.

2.5 Affinity Chromatography

Affinity chromatography was performed to separate anti-histamine antibody from the serum. The immune affinity column preparation was based on Hogesan et al., (1992)[32] and Mohan et al., (1995)[33]. In this method, histamine was conjugated with activated sepharose and chromatographic column was filled as solid phase. After washing histamine+sepharose complex with sodium phosphate buffer, 2M sodium chloride solution was added and equilibrated with sodium phosphate buffer solution. 1 ml of serum was passed through the solid phase to form histamine-antibody complex. This histamine-antibody complex was washed with sodium phosphate buffer and eluted with glycine-HCl pH 2.2 solution and collected in the test tube.

2.6 Protein Estimation

Protein content of the elute was estimated by Bradford assay method (1976)[34] using BSA as a standard.

2.7 SDS-PAGE

Contents of the elute were denatured with 5X Sodium Dodecyl Sulphate (SDS) and resolved by 12% Tris-Gly gel with an appropriate molecular weight marker. Gel was stained by Coomassie blue to visualize the protein bands[35].

2.8 MALDI-TOF

Bands visualized by Coomassie blue were excised and analyzed by MALDI-TOF[36] after tryptic digestion (Indian Institute of Science, Bangalore, India).

2.9 Estimation of SHBC

96-well microtiter plates were coated with 5 µg/100µl/well of histamine (in sodium bicarbonate buffer, pH 9.6) and incubated overnight at 4 °C in the refrigerator. Serum was diluted 1:30000 with distilled water. 100 µl of this diluted serum was added to histamine coated well and incubated for 1 hr. at 37°C. Contents of the well were discarded and washed thrice with wash buffer (Phosphate buffered saline, pH 7.4). Horse radish peroxidase (HRP) conjugated goat anti-human secondary antibody (Sigma) was diluted 1: 50000 with assay buffer. Added 100 µl/well and incubated at 37 °C for 1 hr. Plates were washed thrice, dried and 100 µl of chromogenic agent tetra methyl benzidine (TMB) substrate was added. Wells were incubated for 30 min. at room temperature and reaction was stopped by adding 100 µl/well of stop reagent (2N Sulfuric acid). The plate was read at 450 nm wavelength using an absorbance plate reader (Alere). Serum from a normal person with known protein concentration was diluted in the range of 1:5,000 to 1:70,000 with distilled water. SHBC values were determined and a standard curve was plotted. SHBC of unknown samples were extrapolated using Graphpad prism.

2.10 Treatment with Histaglobulin

These allergic patients were then treated with injection Histaglobulin as per the schedule explained below:

Preparation of patients, with appropriate drugs as explained below, which lasted for 5-7 days followed by Histaglobulin injection as per schedule along with drugs.

2.11 Preparation of patients

Before starting Histaglobulin therapy, patients were administered with the following drugs for 5-7 days: a) anti-histamine drugs Cetirizine or Levocetirizine b) Loratadine or Rupatadine c) Theophylline or Doxophylline d) Monteleukast. Patients were advised to avoid allergic food substances during treatment[37]. Once the allergic reactions including infection, wheezing, watering of nose, sneezing, worm infestation, eosinophilia, and other symptoms were controlled, the patient was considered fit to receive histaglobulin injections.

2.12 Administration of histaglobulin

Histaglobulin was administered subcutaneously in two phases: Phase 1: 6 primary doses at weekly intervals; phase 2: 3 booster doses; first booster dose was given 15 days after the last primary dose; second booster dose at1 month after first booster dose and 3rd booster dose at 2 months after the second booster dose. The dosage of histaglobulin given to different age groups were as follows: Below 1 year up to 5 years - 0.5 ml; above 6 and up to 10 years - 0.75 ml and above 10 years -1 ml. All the drugs mentioned above were continued during six primary doses till 7 days after 1st booster dose and then stopped till second booster dose. The drugs were started again 2 days before 2nd booster dose of histaglobulin injection and continued for 1 week after the injection. The medicines were stopped for 2 months, started again 2 days before third booster dose, continued for one week after third booster dose and then stopped. After completion of the booster doses, total IgE and SHBC were estimated. Patients were reviewed periodically and their wheezing history was followed up routinely.

3 STATISTICAL ANALYSIS

Conversion of SHBC ELISA to arrive at actual protein concentration and statistical analyses were done using Graphpad prism. A statistical significance of $p<0.05$ based on paired t-test was considered significant.

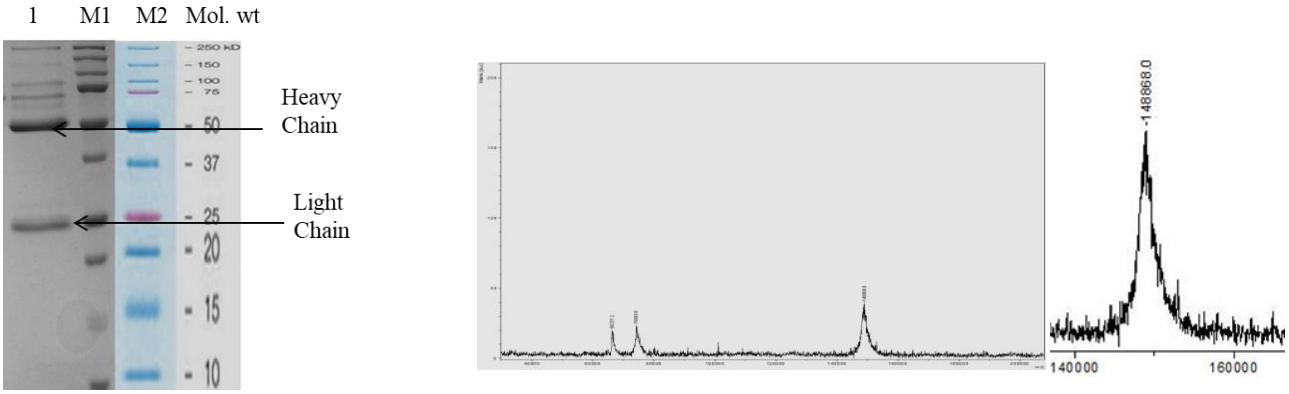
4. RESULTS

4.1 Patient classification

The study was conducted with 67 normal persons and 135 allergic patients. Among 202 persons, 90 persons (45%) were males and 112 persons (55%) were females. Among 135 allergic patients, 62 (45.9%) were males and 73 (54.1%) were females. 45 (33.3%) were from urban and 90 (66.7%) were from rural areas. 90 patients (66.7%) were suffering from bronchial asthma and 40 (29.6%) from allergic rhinitis and 5 (3.7%) from other allergic disorders like cough variant asthma, urticaria and others. Most of the patients were of the age 40 years and above. 16 (11.8%) were suffering from allergic disorders for < 1 year, 77 (57%) were suffering from allergic disorders for 1-6 years, 13 (9.6%) for 7-10 years, 15 (11%) for 11-15 years, 7 (5.2%) for 16-25 years and 7 (5.2%) for more than 26 years. 56 (41.5%) were first sibling to their parents. In 94 patients (69.6%), family history of atopic diseases was present. The prevalence of allergic diseases was noticed in 84 patients (62%) of higher income group. The total Ig E level was below 100 IU/ml in normal persons and more than 200 IU/ml in allergic patients.

4.2 Affinity chromatography and MALDI-TOF

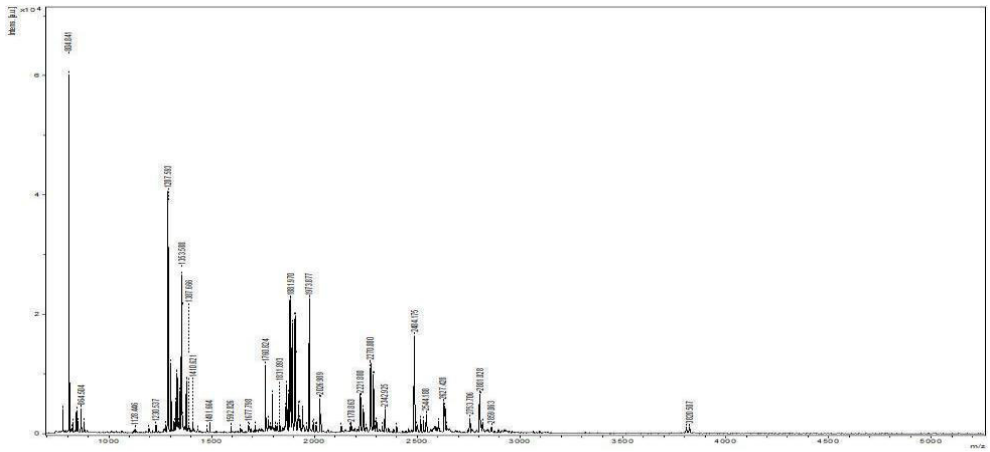
Serum from a normal person was eluted through affinity chromatography. This elute was resolved in 12% SDS-PAGE and the gel was incubated in Coomassie blue. Two clear bands, visualized at ~50 kDa and ~25 kDa (Fig. 1a) were excised and subjected to tryptic digestion. Intact molecular weight estimate from MALDI-MS, 148868 daltons, matched with the expected molecular weight of IgG (Fig. 1b).



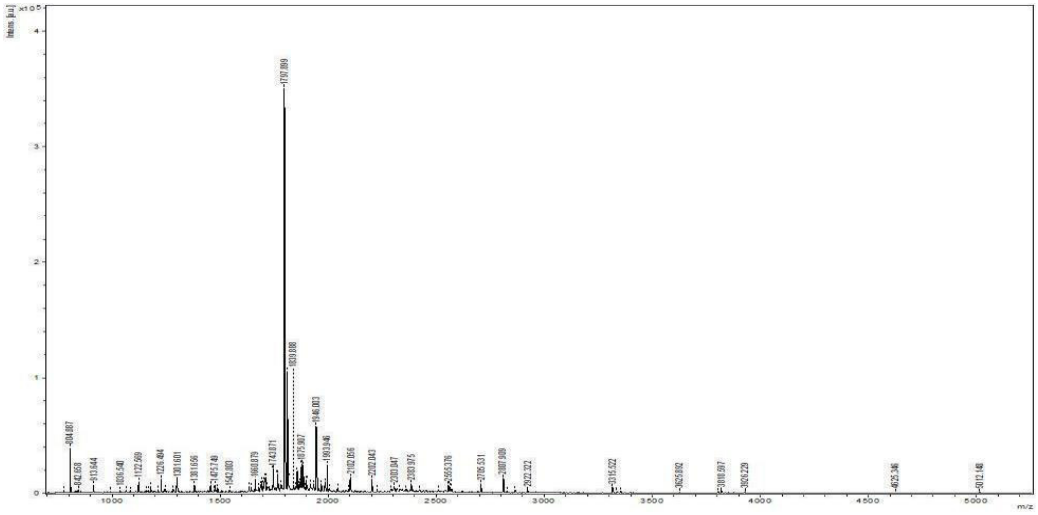
a) Visualization of heavy and light chains SDS-PAGE analysis of serum eluted through affinity chromatography (Lane 1); M1: magic marker; M2: Kaleidoscope marker b) Intact MW estimate from MALD-MS (148868 daltons), as analyzed using
b) UltrafleXtreme MALDI TOF/TOF (Bruker Daltonics).

Fig1. Evaluation of intact molecular weight of 50 and 25 kDa bands from elute.

From MASCOT analysis of MALDI-MS, 50 KDa and 25 KDa regions matched with the IgG heavy chain (Fig. 2a, score: 46, expect 6.4, nominal mass (Mr) 13433 and calculated pI 8.96) and light chain variable regions (Fig 2b, score: 51, expct: 2.2, nominal mass (Mr): 10669 and calculated pI 5.20), respectively. Protein in the sample was found to have the following amino acid sequence corresponding to IgG heavy chain: EVQLLESGGGLVQPGRSLRLSCTASGFTFGDYAMSW RQAPGKGLEWVGFIRRKAYGGTTEYAASVKGRFTISRDD SKSIAYLQMNSLKTEDTAVYYCTRSYGDRHTVDYWGQGT LVTVSS (>gi|12734022|emb|CAC28910.1| immunoglobulin gamma heavy chain variable region, Homo sapiens) and light chain: AASGFRISGFWMSWVRQAPGKGLEWVANIKPDGSEKNYV DSVRGRFTIVRDNAENSLYLQMNSLRDDDTAVYYCARDW PGGSGSSSQDYWGQGTL (>gi|121281345|gb|ABM53262.1| immunoglobulin light chain variable region, Homo sapiens). These studies clearly proved that these bands from the elute corresponded to heavy and light chain of IgG antibody.



(a)

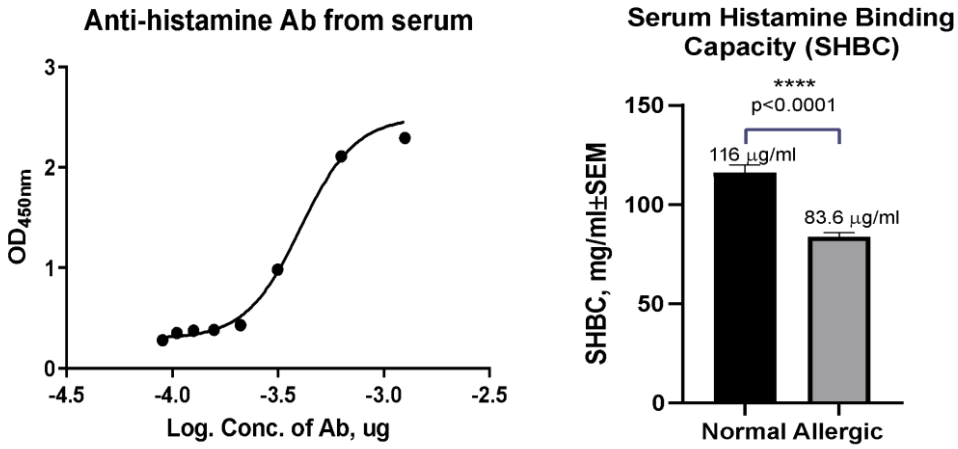


(b)

Fig 2. Tryptic digestion and MASCOT analysis of MALDI-MS. Results of (a) 50 kDa region of gel slice (b) 25 kDa region of gel slice.

4.3 *SHBC and IgE levels*

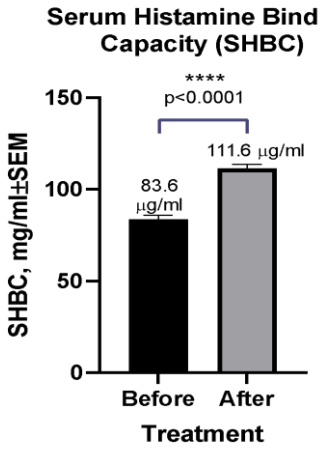
Serum from a normal person was diluted and used to plot standard curve for serum histamine binding capacity (SHBC) ELISA (Fig. 3a). Average SHBC in 67 normal persons was 116 µg/ml while in 135 allergic persons it was 83.6 µg/ml ($p<0.0001$, Figure 3b) suggesting that overall SHBC in allergic persons is ~39% lower than normal persons.



(a) (b)

Fig 3. Serum histamine binding capacity

a) Serum from normal person was diluted from 1:5000 to 1:70,000 and serum histamine binding capacity (SHBC) was determined by ELISA. These values were used to plot a standard curve to extrapolate the SHBC of unknown serum samples from the study. Protein concentration of the normal serum was determined by Bradford method. b) SHBC level was determined in 67 normal and 135 allergic patients by ELISA and the average SHBC±SEM (µg/ml) was plotted. Statistical difference between the data set was assessed to be $P<0.0001$ by t-test.



SHBC level of 135 allergic patients was determined by ELISA before and after treatment with Histaglobulin therapy and the average SHBC±SEM (µg/ml) was plotted. Statistical difference between the data set was assessed to be P<0.0001 by t-test.

Fig 4. Serum histamine binding capacity in allergic patients before and after treatment.

Among the 135 patients, the average SHBC was raised to 111.6 in 123 patients (91.1%, Fig. 4) and lowered in 12 (8.8%) patients. In 82 patients (60.7%) the SHBC level was raised and total IgE level was lowered. In 41 patients (30.4%) the levels of both SHBC and total IgE were raised. In 7 patients (5.2%) both IgE and SHBC levels were lowered. One patient had wheezing on stopping the treatment. In 5 patients (3.7%) the total IgE level was raised and the SHBC level was lowered. Among these 5 patients, 2 patients had recurrence of allergic manifestations. The reason for rise in total IgE level after treatment may be due to exposure of the patient to an allergen such as pollen grain, dust mite etc. or a food item that was not tested earlier. Overall, 132 patients (97.8%) responded well and were free from allergic manifestations and histaglobulin treatment was tolerated well without any adverse side effects.

5. DISCUSSION

Although people from urban, rural and industrial areas are exposed to similar allergens, only 10-15% of them manifest allergic reactions while 85-90% of population remains unaffected[38]. Therefore, it is reasonable to assume that the causative factor determining sensitivity to allergens remains within the person. Accordingly, it has been reported earlier (Parrot et al., 1953)[29] that serum histamine binding capacity (SHBC) of people prone to develop allergic reactions is lower as compared to people who are not prone to allergies. Our study clearly shows that the SHBC is ~39% lower in allergic patients. Therefore, we hypothesized that if we can increase the SHBC in such people, we should be able to inhibit the allergic manifestations. We had reported earlier that Histaglobulin treatment, as administered by us to 161 patients resulted in complete and durable diseases control in 152 of patients (94.6%), not only against bronchial asthma, but also against other allergic conditions such as allergic rhinitis and others[30]. In our study, we had followed up the patients for about 15 years after treatment where they were found to be asymptomatic and free of clinically measurable disease. According to Gelfand[39] who studied Evaluation of Histamine Gammaglobulin (Histaglobulin) in the treatment of various allergic conditions, 'Histaglobulin' in some way, as yet unexplained, altered the allergic state in a significant proportion of patients, suffering from bronchial asthma, allergic rhinitis, etc . Tanaka et al., (1975)[40] based on their clinical research, concluded that the effects of histaglobulin was remarkable in children aged 7 and less and that Histaglobulin could be used broadly in other allergic diseases. Satpathy et al[41], Yamamoto et al[42], Asokan et al[43], and Matsumara et al[44] have reported on the benefit of using Histaglobulin against bronchial asthma and other allergic conditions such as allergic rhinitis and chronic urticaria[45, 46]. Apart from enhancing the level of antihistamine antibody and thereby enhancing serum histamine binding capacity, it has been reported that anti allergic drug Histaglobulin inhibits NF-Kappa B nuclear translocation and down- regulates pro inflammatory cytokines[47]. Parrot et al., (1958) reported that normal persons have 25-30% higher histamine binding power than allergic individuals as assessed by histaminophylaxis[48]. Histaglobulin injection was developed using histamine as a hapten and globulin, a large immunogenic protein as a carrier[49]. When histaglobulin was injected subcutaneously into guinea pig, it caused the animal to develop resistance to the inhalation of a histamine aerosol. This came on in about six weeks and lasted for the rest of the life of the animal. Based upon Karl-Landsteiner's study on haptens in 1920-1930s that, when a hapten was conjugated with a carrier such as albumin or globulin and injected subcutaneously, the hapten-carrier complex elicited 3 types of antibodies, namely a) antibody to hapten conjugate which constitutes a major portion b) antibody to carrier and c) antibody to hapten carrier complex[49]. Accordingly, histaglobulin injection was developed by Parrot and her co- workers, using histamine as a hapten and globulin, a large immunogenic protein as a carrier. Apart from eliciting antibody formation, histaglobulin injection also leads to the production of memory cells, B and T lymphocytes, thereby causing long lasting immunity. Therefore, by administering histaglobulin injection subcutaneously, antibody to histamine can be increased which enhances the serum histamine binding capacity (SHBC) of the individuals and provides lifelong protection from allergens that a person was previously exposed to.Histaglobulin is being used in clinic by various groups for the treatment of multiple allergic diseases[50-54]. Jankowska's study on the influence of histaglobulin therapy in patients with atopical bronchial asthma[50], Kaur's study of clinical efficiency and safety of histaglobulin and fexofenadine in patients with chronic idiopathic urticaria[51], Mohanty's work on prevention of multiple drug allergy by Histaglobulin[53] and Noh's work on immunotherapy using Histaglobulin in atopic dermatitis[54] seem to have limited clinical efficacy, with some early remission. But, so far it has not been studied in detail

how Histaglobulin acts in various allergic conditions such as chronic bronchial asthma, allergic rhinitis, urticarial, to bring about temporary relief or remission. Till now, there is no consensus on how Histaglobulin should be used to maximize efficacy. In our research work of more than 20 years, we have standardized the dosage, duration and time of treatment initiation with Histaglobulin. We hypothesize that since the allergic pathways are already activated and histamine receptor is sensitized, histaglobulin injection is not highly efficient in evoking significant antibody response in these individuals. If these allergic reactions are well controlled before the administration of histaglobulin, we should be able to evoke stronger response. Therefore, in our treatment method, patients were first prepared by administering with several medicines to counteract the action of histamine, to stabilize the mast cell and antagonize both H1 histamine receptor and platelet activation factor (PAF) receptors, to stabilize mast cell membrane by inhibiting the enzyme phosphodiesterase thereby increasing cAMP and to reduce the inflammatory effects of leukotrienes. Therefore, by administering the drugs mentioned earlier, the mast cell membrane was stabilized, histamine released from mast cells was counteracted and all allergic reactions were thus controlled before histaglobulin therapy. Patients were also advised to avoid exposure to identified allergic food and environmental allergens during the treatment. Then histaglobulin injection was given as per schedule mentioned earlier. Since normal persons have higher histamine binding capacity, all the histamine released upon exposure to an allergen is neutralized and there are no allergic manifestations. In an allergic patient, there is still some histamine left to act upon the H1 receptors of target cells thereby causing allergic symptoms. Accordingly, results of our study with 67 normal and 135 allergic persons proved that the SHBC of normal people was ~39 % more as compared to allergic people. Further, SHBC level was raised by an average of ~33% in allergic patients after treatment and 132 (97.8%) persons became free from allergic manifestations.

6. CONCLUSION

From our study, we clearly demonstrated that SHBC was higher in normal persons than in allergic persons. After treatment with our novel histaglobulin treatment, SHBC level increased significantly with a concomitant decrease in total IgE level in most cases. 132 out of 135 allergic patients were completely relieved from allergic manifestations. Since this method of treatment with histaglobulin which has a strong scientific basis, effective, safe and well tolerated, it can be adopted across the world for effecting a cure from bronchial asthma and allergic rhinitis.

7. AUTHOR'S CONTRIBUTION

K. Sivanandhan: Original hypothesis, clinical treatment, data acquisition, drafting of manuscript. S. Dhanalakshmi: Concept and design of study, data analysis, statistical analysis and manuscript editing and revision.

8. CONFLICT OF INTEREST

Conflict of interest declared none.

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