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Leukocyte Adherence Inhibition Test in Evaluation of Non-IgE-Mediated Immunoreactivity to Potassium Sorbate

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ABSTRACT

Background & Objective: Non-IgE (Type IV) hypersensitivity is a complex issue for allergic patients with no proper diagnosis and treatment. The Leukocyte Adherence Inhibition Test (LAIT) measures antigen-specific, cell-mediated immune responses by detecting the inhibition of leukocyte adherence to glass surfaces upon exposure to an antigen. This process is mediated by the release of lymphokines such as Leukocyte Migration Inhibition Factor (LIF). This makes it a functional assay for non-IgE hypersensitivity detection. This study aimed to use LAIT in detection of non-IgE-mediated immunoreactivity to potassium sorbate.

Materials & Methods: This retrospective study involved a review of 50 patients' medical history with diagnosis of various allergic phenotypes; including allergic bronchitis, sinus headache, and urticaria and who were suspected of having non-IgE-mediated hypersensitivity to potassium sorbate. An *ex vivo* challenge test was done by incubating the patients' leukocytes with potassium sorbate, and the resultant immunoreactivity was measured by LAIT, with major readout of the extent of leukocyte adherence inhibition (LAI).

Results: The findings indicated that the values of LAI were very much varied with a minimum and maximum of 0 and 98, respectively, and the mean, median, and standard deviation of 52.2, 51.6, and 18.9, respectively. The distribution of LAI results as a cascade revealed patients with mild, moderate, or severe non-IgE-mediated immunoreactivity to potassium sorbate. Others did not show any immunoreactivity. These preliminary results indicate LAIT that worked with potassium sorbate can possibly select different levels of *ex vivo* immunoreactivity of patients with various allergic phenotypes.

Conclusion: This research offers information about the potential usefulness of LAIT in measuring non-IgE-based immunoreactivity against potassium sorbate. The findings may be priceless in knowing the reason behind non-IgE-mediated allergic reactions mechanisms and possibly guiding the potential creation of more individualized treatment methods for these patients.

Keywords: Potassium sorbate, Leukocyte Adherence Inhibition Test, LAIT, Non-IgE-mediated hypersensitivity

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1. Introduction

Potassium sorbate provides a source of potassium ions; an essential dietary mineral, which contribute to the body's supply of this vital electrolyte. Potassium sorbate with following chemical formula: C6H7KO2 composed of carbon, hydrogen, potassium, and oxygen is the potassium salt of the organic compound sorbic acid (1). It

is used as preservative and additive ingredient, with controlled level by law, in industrialized food, cosmetics, and medications as it has activity against moulds, yeasts, and certain bacteria in food, drinks, cosmetics, and pharmaceuticals (2-4).

Potassium sorbate and its similar compounds give valuable background to evaluation of the potential of leukocyte adherence. The inhibition test was used to differentiate non-IgE-mediated immunoreactivity with potassium sorbate in patients having different allergic phenotypes (5). Potassium sorbate is hydrolyzed in exposure to water and releases sorbic acid and potassium. The widespread availability of potassium sorbate and its derivatives in human diet may imply their extensive exposure to the immune system (6, 7). Potassium sorbate and other food additives have long been recognized as potential sensitizing agents capable of triggering human allergic reactions. There have been some studies that found potassium sorbate elicited objective reactions in 7% of the patients with urticarial reactions (5, 8).

Leukocyte Adherence Inhibition Test (LAIT) is a developed *ex vivo* test that assists in identifying antigen-specific, cell-mediated (Type IV) hypersensitivity. Its mechanism is the inhibition of leukocyte adherence to a glass surface after antigen exposure and this process is carried out by the release of lymphokines like Leukocyte Migration Inhibition Factor (LIF) by the sensitized T-lymphocytes. This renders it an appropriate practical instrument to query non-IgE-mediated immunoreactivity (9). There have been reports of a child developing chronic cheilitis after ingesting industrialized food preserved with potassium ions. Asthmatic patients have experienced bronchospasm following the intake of potassium-contained anti-asthmatic medications. The immunoreactivity elicited against potassium sorbate does not usually manifest as an immediate, rapid reaction (like a classic IgE-mediated allergy (10). The delayed nature of the reaction suggests that the involved immune mechanism is not a classic IgE-mediated allergy (10, 11). The key insights into the mechanism of potassium ions (and similar food additive) hypersensitivity have been provided by *ex vivo* leukocyte challenge tests, which have demonstrated an increase in sulfidoleukotriene production (12). Since LAIT detects non-IgE-mediated immunoreactivity by measuring leukotriene-driven inhibition of leukocyte adherence, it can therefore serve as an aid in identifying hypersensitivity to potassium sorbate (13, 14).

It provides a feasible alternative to more exhaustive provocation testing (15). To assess the ability of LAIT to identify non IgE-mediated immune response toward benzoic acid, the electronic medical records of patients who had been evaluated with this test were retrospectively reviewed. The patients had been diagnosed with non-IgE-mediated conditions such as eczema, asthma, allergic bronchitis, sinus headache, allergic rhinitis, and /or urticarial reactions (16, 17).

Potassium sorbate was chosen as the antigen of interest in this study because of its ubiquitous use as a preservative supplement in a highly diverse range of processed food, drinks, and personal care products, exposing the population to its effects almost daily. Although categorized under the generally safe group, it has been associated with a variety of clinical reports and case studies with adverse reactions, such as urticaria, angioedema, and respiratory symptoms in a subgroup of people. The underlying pathogenic processes of these reactions are however poorly delimited and are commonly called intolerance.

The hypothesis that is being examined in the given study is that a certain part of these reactions is caused by a particular, immune-mediated hypersensitivity, and not a non-immune pharmacological effect (18). The LAIT has been utilized successfully to measure non-IgE-mediated immunoreactivity in patients with different allergic phenotypes to a variety of triggers, including mixed aeroallergens and food allergens (10), and specific low-molecular-weight compounds, such as benzoic acid and polyethylene glycol (19).

Potassium sorbate is a preservative that is ubiquitous in food, beverages, and pharmaceuticals. Although it is widely considered to be harmless, it has been associated with cutaneous adverse reactions, and it has been postulated that contact sensitization is possibly under-identified (20). Its possible capability to stimulate systemic, non-IgE-mediated immunoreactivity that contributes to more general allergic phenotypes is however, not well characterized.

The current study was designed to estimate the Leukocyte Adherence Inhibition Test ability to differentiate varying degrees of non-IgE-mediated immunoreactivity against potassium sorbate among patients with different allergic reactions. We utilized LAIT to investigate and quantify the *ex vivo*, non-IgE-mediated immunoreactivity to potassium sorbate in a cohort of patients with diagnosed allergic conditions.

2. Materials and Methods

2.1 Subjects

Ethical approval was obtained from the Institutional Review Board in College of Medicine, University of Kerbala. The study proceeded with an electronic chart review of 900 outpatients who attended their facility from October 2023 to June 2024. From this broad patient population, group of 50 patients were subjected to an *ex vivo* allergen challenge test. For this purpose, peripheral blood mononuclear cells (PBMCs) were incubated with potassium sorbate (1 mg/mL). Antigen-specific, cell-mediated immunoreactivity was then measured by the Leukocyte Adherence Inhibition Test (LAIT). These patients had been diagnosed with non-IgE-mediated conditions such as allergic bronchitis, allergic rhinitis, skin conditions like atopic dermatitis, and/or urticarial reactions and asthma.

The sample size of the study was 20 individuals. The mean age was 30.5 ± 12.7 years (maximum: 86 years;

median: 38 years). The patients were chosen on clinical suspicion of hypersensitivity to potassium sorbate with an inconclusive or non-reactive skin prick test to potassium sorbate hence acted as a control group of non-IgE-mediated reactivity.

2.2 Inclusion Criteria

The diagnostic criteria for patients inclusion were based on: 1) typical symptoms recurring with suspected food intake; 2) negative standard diagnostic workup for IgE-mediated allergy (skin prick tests and/or serum specific IgE to common food and inhalant allergens); and 3) lack of response to standard anti-IgE or antihistamine therapies where applicable.

2.3 Exclusion Criteria

Patients with active infection, immunosuppression, or other systemic inflammatory diseases were excluded. The study did not include pregnant women, breastfeeding, and patients under systemic anti-inflammatory therapy and/or biological therapy (corticoids, cyclosporin) as well.

2.4 Leukocyte Adherence Inhibition Test

The study conducted the Lymphocyte Activation Immunotest (LAIT) using a previously established protocol (18). Briefly, each participant's fresh plasma was divided into two aliquots. One aliquot was subjected to *ex vivo* challenge test with a 1 mg/mL solution of potassium sorbate, while other aliquot was left unchallenged to serve as a control. To prepare the samples, first the buffy coat fraction, which contains a high concentration of leukocytes, was isolated from the heparinized blood after allowing it to sediment for one hour at 37°C, then, 100 µL aliquots of the buffy coat was distributed into separate microcenterfuge tubes. For the challenged samples, 10 µL of potassium sorbate solution (1 mg/mL, pH 7.5) was added to the tubes and incubated under agitation (200 rpm at 37°C) for 30 minutes. The control sample was incubated without potassium sorbate addition. After the incubation period, the plasma samples were transferred into a standard hemocytometer count chambers with non-metallic glass surface. The chamber was then placed in water bath at 37°C for 2 hours, allowing the leukocytes to adhere to the glass surface. In the following, the number of leukocytes adhered to the glass surface of the Neubauer hemocytometer chamber were counted. Then, the coverslip was carefully separated and the chamber was washed by fully immersing in a beaker filled with phosphate buffer saline (PBS) at 37°C. Finally, a fresh drop of PBS was added to the chamber and a clean coverslip was placed over it. Then, the remained adherent leukocytes were re-counted in the same squares that were examined accordingly.

2.5 Leukocyte Adherence (LA) Percent

The numbers of leukocytes were counted on hemocytometer chamber glass surface before and after washing. The leukocyte adherence (LA) percentage was determined by dividing the number of leukocytes that were left after washing by the first number before washing and multiplying the result by 100. This provided us with the percentage of leukocytes that could resist washing and remained clapped to the glass. Lastly, the leukocytes adherence ratio (LAR) were measured by dividing the antigen-challenged sample percentage of LA by the LA percentage of unchallenged control sample, and, thereafter, multiplying the result by 100. The LAI was determined by comparing the adherence of leukocytes in the challenged condition against the control condition.

The percentage of inhibition was calculated using the formula: LAI (%) = 100 – LAR (challenged).

2.6 Statistical Analysis

The results of Leukocyte Adherence Index (LAI) were used to create a cascade distribution chart. Also, the LAI data was used for the statistical calculations with Microsoft Excel (version 2020) software package to calculate mean, standard deviation, and standard errors.

3. Result

Spectrum of Immunoreactivity and Threshold Establishment

The *ex vivo* immunoreactivity to potassium sorbate, as measured by LAIT and captured in the digital medical records of the patients, showed a broad distribution among 50 patients. Descriptive statistics for the Leukocyte Adherence Index (LAI) are presented in Table 1. The wide range of LAI values (0 to 98) and the standard deviation (18.9) relative to the mean (52.2) indicate considerable heterogeneity in immunoreactivity within the cohort. As seen in the cascade distribution chart (Table 2), there is a wide variation in LAI results. Four patients exhibited LAI values indistinguishable from or rounded to 0%, indicating that leukocyte adherence following potassium sorbate exposure was quantitatively equivalent to the baseline control measurement for those individuals. To move beyond descriptive statistics and define clinically relevant categories, immunoreactivity was stratified into three levels based on the distribution of LAI values and biological plausibility, a method consistent with prior LAIT studies on food additives. The cascade distribution of the cohort (Figure 1) informed the following thresholds: Low/Negligible Reactivity: LAI ≤ 20%; this group (n=10, 20%) largely represented the background or non-specific response, Moderate Reactivity: LAI 21-50%; this was the most populous group (n=29, 58%), capturing the central tendency of the cohort immune response and high Reactivity: LAI > 50%; this group (n=11, 22%) represented patients with a pronounced *ex vivo* immunoreactivity.

As seen in the cascade distribution chart (Table 2) there was a wide variation in results of Leukocyte Adherence Index (LAI). Four of the patients had no inhibition of leukocyte adherence (LAI = 0) following exposure of potassium sorbate, which implies a total absence of reaction to the allergen (2% of the total tests). Other participants demonstrated lower or moderate immunoreactivity to the *ex vivo* challenge test. The rest of

individuals demonstrated strong immunoreactivity. This wide variation in responses could potentially suggest the involvement of potassium sorbate in a non-IgE-mediated hypersensitivity condition, which would require further corroboration through *in vivo* provocation testing (Figure 1).

Table 1. Leukocyte adherence index among study population.

Test	N	Mean	Median	Standard Deviation	Minimum	Maximum
Leukocyte Adherence Index (LAI)	50	52.2	51.6	18.9	0	98

Table 2. Cascade distribution of Leukocyte Adherence Inhibition (LAI) range groups.

Number of tests/subjects	Percentage
LAI 0%	2
1-20%	8
21-40%	14
41-50%	15
51-80%	7
81-98%	4

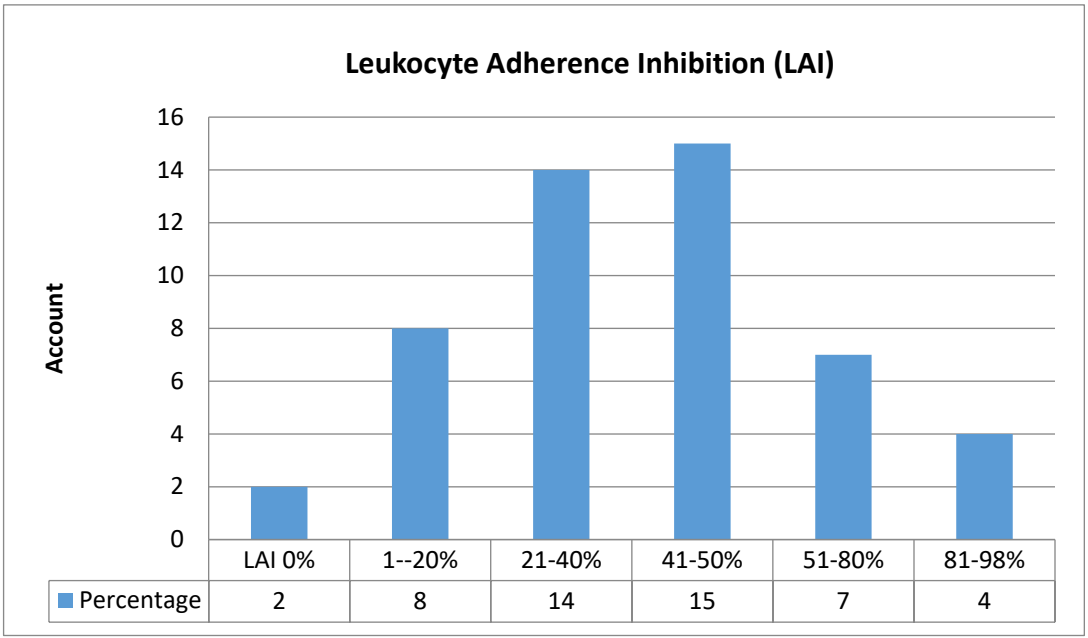

Figure 1. Cascade distribution chart of Leukocyte Adherence Inhibition (LAI) range groups (**Prepared by Authors, 2026**).

4. Discussion

This study showed a broad range of *ex vivo* leukocyte reactivity to potassium sorbate using the LAIT. Although the LAIT is a validated cell-mediated (Type IV) hypersensitivity detection method, as it is applied to allergens and other food additives **(21)**, we admit that the adherence inhibition may be due to non-specific activation of leukocytes, or direct pharmacologic influence on cellular adhesion molecules. Such a pure pharmacologic effect would presumably give more consistent response across all samples rather than the diffuse, patient-specific distribution (LAI 0-98%), which is typical of an adaptive immune memory response **(18)**. Moreover, dividing of the patients into reactivity groups was associated with different clinical phenotypes. The diagnosing of non-IgE-mediated hypersensitivity conditions is technically challenging due to the lack of

standardized immunoassays for these types of reactions (18). Identifying these conditions among patients with allergies relies on a laborious, individualized clinical approach. This involves conducting a thorough medical history, performing cutaneous tests, and carrying out *in vivo* provocation challenges - all of which are done after meticulous elimination diets to rule out other potential triggers (19). Hypersensitivity to benzoic acid may, in a certain way, be similar to and potentially enhance the hypersensitivity produced by non-steroidal anti-inflammatory drug (NSAIDs). This is because potassium sorbate, as an increaser of sulfidoleukotriene production, can have a mechanism of action that is in some ways analogous to the principal pharmacological action of NSAIDs (20). The primary modes of NSAID actions are inhibition of cyclooxygenase (COX) enzyme, which catalyzes the synthesis of prostaglandin and thromboxanes. By interfering with this pathway, NSAIDs can lead to hypersensitivity reactions in some individuals (21). Similarly, the increased production of sulfidoleukotrienes induced by potassium sorbate exposure may trigger hypersensitivity responses that share certain similarities with hypersensitivity seen with NSAID use. In this way, potassium sorbate could potentially enhance or exacerbate the hypersensitivity effects caused by NSAIDs, as both compounds can disrupt the balance of inflammatory mediators in the body (22). The pharmacologic inhibition of COX enzymes leads to an increase in the activity of lipoxygenase (LOX) pathway. This, subsequently, leads to increased leukotrienes production. The balance of autacifs (local hormones) in the body can be disrupted by any substance, pharmacologically or immunologically, that amplifies leukotrienes production. The negative change in the autacoid balance may lead to the allergic symptoms appearance (23).

LAIT is based on the assumption of supplying *ex vivo* modelizing the immune response. The assay enables the combined interaction of several cell parts of the immune system by stimulating a viable buffy coat leukocyte sample with a particular antigen, hence quantifying an active immunologic event (24). However, as an observer of the final phenomenon, the LAIT does not provide information on which specific pathway was involved in inhibiting leukocyte adherence or increasing the production of leukotrienes, whether through pharmacological or immunological mechanisms (25). The *ex vivo* LAIT challenge of benzoic acid resulted in a broad span of outcomes in this initial retrospective cohort of diverse phenotypical allergic patients (26). Regularly, we use the LAIT as an adjunctive triage test to inform the choice of antigens on which to *in vivo* provocation tests are to be used in a more complex manner, a strategy that is especially useful when individual IgE levels cannot be measured (27). Notably, none of our patients exhibited an exclusive reaction solely to potassium sorbate. Instead, each patient was simultaneously tested with a panel of protein allergens (e.g., mites, fungi, food allergens), and some of these protein allergens also elicited positive results (28). These findings suggest that allergic patients may experience an exacerbation of their symptoms due to a pharmacological or immune-mediated additional effect of potassium sorbate (29, 30) over and above the primary hypersensitivity response (31).

5. Conclusion

In conclusion, this retrospective exploratory study gives preliminary evidence on *ex vivo* leukocyte reactivity to potassium sorbate in a cohort of patients of different allergic phenotypes as assessed by LAIT. The wide range of LAI values observed indicate that immunoreactivity is heterogeneous and this may indicate a non-IgE-mediated component in a subset of individuals. Nevertheless, these results are early, and should be viewed with reservations. Lack of a prospective control group and confirmatory *in vivo* challenges prohibits any definite claim and conclusion of clinical causality at this stage. Therefore, this effort should be considered hypothesis-generating.

6. Declarations

6.1 Acknowledgments

The authors would like to thank the staff of the College of Nursing, University of Al-Qadisiyah, for their cooperation and technical support during sample collection and laboratory procedures. Appreciation is also extended to all participants for their willingness to take part in this study.

6.2 Ethical Considerations

The study was approved by the Ethical Committee. Ethical approval was obtained from the Institutional Review Board (IRB) of University of Kerbala (Approval No. REC2024.11.27, dated November 27, 2024). Written informed consent was obtained from all participants prior to their inclusion in the study.

6.3 Authors' Contributions

Ihsan K. A. Alkardhi: Conceptualization, methodology, data collection, laboratory work, data analysis, writing—original draft, corresponding author. Hasanain Riyadh Al-Isawi: Supervision, methodology, validation, review and editing. Suha Allawi Hussein Almrumudhe: Laboratory support, data interpretation, manuscript review. Hayder Ali Muhammed: Data analysis, scientific input, manuscript review and editing. All authors reviewed, edited, and approved the final version of the manuscript.

6.4 Conflict of Interest

The authors declare that they have no conflicts of interest relevant to this study.

6.5 Fund or Financial Support

The study was fully self-funded by the authors.

6.6 Using Artificial Intelligence Tools (AI Tools)

Artificial intelligence tools (ChatGPT, OpenAI) were used solely for language polishing, grammar correction, and formatting guidance. No AI tool was used for data generation, statistical analysis, interpretation of results, or drawing scientific conclusions. All scientific content, data, and interpretations were produced entirely by the authors.

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