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Investigation of alkaline phosphatase activity and obesity as biological markers in asthma patients in Babylon governorate

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Abstract

Background: Asthma inflammation results from the activation of several components that generate pro-inflammatory cytokines. Corticosteroid therapy can affect bone formation by altering the activity of the alkaline phosphatase enzyme. Although the causes of this link are unclear, obesity is also a significant risk factor for developing asthma. It is thought that inflammatory alterations in adipose tissue may cause increased airway inflammation and responsiveness in obese people.

Objectives: This study aimed to evaluate an increase in alkaline phosphatase due to inflammatory events and study the effects of obesity on asthma.

Patients and methods: There were one hundred participants in this case-control study. The blood samples were collected from the allergy and asthma center in Hilla City and split into two groups: the control group and the patient group, which had asthma symptoms.

Result: Significant gender differences were observed in the asthma group, which had a higher proportion of women than men. Regarding the relationship between asthma and blood group, our result showed that among patients with asthma, the A+ group was the most common blood group by 35%. For body mass index results, it was noted that individuals who are obese are more prone to asthma. Serum alkaline phosphatase activity was found to be significantly higher in the asthmatic groups than in healthy controls.

Conclusion: This study found significant gender differences in asthma prevalence, with more women affected than men. Severity levels were higher than the global average of 5%, and the severe category had the fewest cases. Asthma was more common in individuals with A+ blood type, and obese individuals showed significantly higher rates of asthma. Notably, obese women had higher asthma rates than obese men. Additionally, serum alkaline phosphatase activity was raised considerably in asthmatic participants compared to healthy controls.

Keywords: Asthma; Asthma severity; Alkaline phosphatase; Obesity; Body mass index

1. Introduction

A chronic inflammatory disease of the airways, asthma affects many different cells and parts. Mast cells, eosinophils, and neutrophils are important participants in this process, especially in smokers, occupational asthma patients, and during abrupt, acute exacerbations. Furthermore, the illness is exacerbated by T lymphocytes, macrophages, and epithelial cells [1,2]. About 300 million people worldwide have asthma, which is a serious global health concern that claims roughly 1,000 lives every day. Most of these deaths are avoidable, and the majority occur in lower- and medium-income nations [3]. Wheezing, shortness of breath, coughing, and a constricted chest are all symptoms of asthma.

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Asthma symptoms are brought on by inflammation of the airways, which sets off reactions such as mucus production, airway wall remodeling, and bronchial hyperresponsiveness (BHR). BHR is the term used to describe smooth muscle cells' propensity to overreact to vague stimuli, such as chilly air. [4,5]. Childhood-onset asthma is the term used to describe asthma that can start in childhood. However, some people may get late-onset asthma, which is a condition that manifests later in life. There are numerous differences between these two forms of asthma. Late-onset asthma tends to be more intense and is less often related to allergies compared to childhood-onset asthma. For children, key risk factors for the persistence of asthma include atopy, reduced lung function, and respiratory tract disorders, especially those caused by rhinovirus [6,7]. The burden of asthma is considerable, affecting quality of life, productivity, healthcare costs, and increasing the risk of hospitalization or death. While asthma cannot be cured, effective management can help control the disease, prevent its progression, and even reverse symptoms, allowing individuals to lead a fulfilling life [8]. The Global Strategy for Asthma Management and Prevention states that there are five distinct clinical phenotypes of asthma.

- **First, Allergic asthma:** Allergy asthma, the most common kind of asthma, typically initially appears in children. It is often associated with a personal or family history of allergic diseases, including eczema, allergic rhinitis, or sensitivities to foods and medications. Eosinophilic airway inflammation is frequently observed in the provoked sputum of these individuals prior to treatment. Inhaled corticosteroid (ICS) therapy usually works effectively for patients with this kind of asthma [9,10].
- **Second, Non-allergic asthma:** Some people suffer from asthma that is unrelated to allergies. These individuals may have neutrophilic, eosinophilic, or paucigranulocytic (having few inflammatory cells) sputum cells. A lower short-term response to ICS is frequently observed in patients with asthma that is not allergic [11].
- **Third, Adult-onset (late-onset) asthma:** happens when people, particularly women, develop asthma for the first time in adulthood. These patients commonly do not have allergies and may need higher doses of inhaled corticosteroids (ICS) or may be less responsive to corticosteroid therapy. It is essential to rule out occupational asthma, which is asthma triggered by work-related exposures, in patients diagnosed with adult-onset asthma [12].
- **Fourth, Asthma with persistent airflow limitation:** this condition, which is frequently not entirely reversible, affects certain people who have had asthma for a long time. It is thought that remodeling of the airway walls causes this condition [13].
- **Fifth, asthma with obesity:** Some obese asthmatic patients exhibit significant respiratory signs along with a distinct pattern of airway inflammation that is characterized by little eosinophilic inflammation [14].

Corticosteroids are used to treat inflammatory conditions. However, inhaled corticosteroids can cause changes in bone, including osteoporosis, thickness, and strength, in asthmatic individuals. It is distinguished by reduced activity of the blood circulation enzyme alkaline phosphatase and affects around 55% of asthmatic patients taking corticosteroids. By increasing inorganic phosphate and encouraging bone mineralization, ALP is known to contribute to bone development. Damaged or decreased bone growth is linked to decreases in these enzymes' activity [15,16].

Alkaline phosphatase (ALP), discovered in 1907, is a glycoprotein linked to plasma membranes that catalyzes the hydrolysis of various monophosphate esters at an ideal alkaline pH, releasing inorganic phosphates [17]. Alkaline phosphatase (ALP) [EC 3.1.3.1] enzyme is encoded by distinct genes as many tissue-specific isozymes. It is found in various organisms (animals, bacteria, and plants) [18]. Alkaline phosphatase is abundant in the stomach, liver, bone, kidney, placenta, and other tissues. The liver, skeletal, and renal tissues are the primary origins of serum ALP [19].

Obesity is a significant risk factor for asthma in both adults and children. Although a threshold BMI is used to define obesity, new research indicates that BMI z-scores may not always be accurate, especially for children and adolescents who are very obese [20]. Adults are deemed obese if their body mass index is thirty kilograms per square meter or above; however, a particular BMI can indicate a wide range of physiological and metabolic health [21]. This differential is probably significant for asthma because, although serum interleukin (IL-6), which is a measure of metabolic health and is generated by macrophages in adipose tissue, is a marker of the severity of asthma, some people with non-obese BMIs have raised IL-6 [22]. According to Sideleva et al., obese people with asthma had greater levels of adipose tissue inflammation than obese controls [23].

The purpose of this study was to show how ALP enzyme activity tests may be used to assess the biological alterations in bone formation brought on by inflammation in asthma and to ascertain if obesity and asthma are related.

2. Material and methods

2.1. Study Settings

This study was conducted on patients who attended the allergy and asthma center in Babylon Governorate in the city of Hilla.

2.2. Study Design

The type of study is a case-control study that compares the control and patient groups.

2.3. Study Population

This study included 100 samples from blood samples, and the age of participants ranged from 22 to 64 years. Blood samples were divided into the following groups:

- Control Groups: Collected from adults with no asthma symptoms.
- Patient Groups: Collected from patients with asthma symptoms.

2.4. Data Collection

2.4.1. Inclusion Criteria

The socio-demographic characteristics used to select the study individuals included patients with asthma. Other parameters recorded in the patient questionnaire are age, blood group, education, waist, hip, type of disease, symptoms that appear in the person, height, and weight.

2.4.2. Exclusion Criteria

Any subject suffering from the following diseases: patients with diabetes, pregnant women, and other lung diseases.

2.5. Collection of Samples

All individuals were seated with a tourniquet and had venous blood samples taken using a disposable syringe (3 mL). After being collected, the blood was carefully mixed in the Jel tube. After that, it was centrifuged for around ten minutes at 3000 RPM. The serum was centrifuged, then moved to a plain tube, and kept at -20°C until analysis.

2.6. Body Mass Index (BMI):

The ratio of an person's weight to height is known as their body mass index, and it is frequently used to classify weight as either healthy or unhealthy. BMI is calculated as follows:

$$\text{BMI}(\text{Kg}/\text{m}^2) = \frac{\text{Weight (Kg)}}{(\text{Height})^2 \text{ m}^2}$$

BMI is the unit of measurement used to describe the global obesity epidemic. It was created with epidemiologists' ease in mind. Table 1 displays the BMI classes [24].

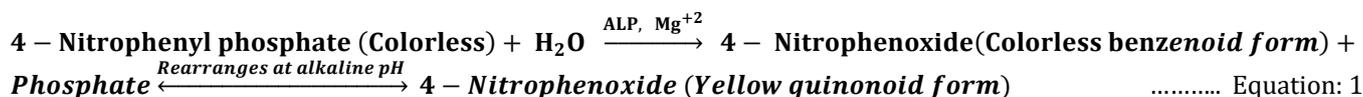
Table 1 Body Mass Index Classification according to the World Health Organization

| No. | Body Mass Index | Weight Status |
|-----|-----------------------|---------------------|
| 1. | Less than 18.5 | Under weight |
| 2. | 18.5-25 | Normal weight |
| 3. | More or equal than 25 | Over weight |
| 4. | More or equal than 30 | Obesity |
| 5. | 30-34.9 | Class one obesity |
| 6. | 35-39.9 | Class two obesity |
| 7. | More or equal than 40 | Class three obesity |

2.7. Determination Activity of Alkaline Phosphatase (ALP) by Biosystem Kit

2.7.1. Principle

Alkaline phosphatase (ALP) functions at an alkaline pH to hydrolyze nitrophenyl phosphate; p-nitrophenol and phosphate are produced as a result. An increase in absorbance, which is directly proportional to the ALP activity in the sample, is used to assess the rate of p-nitrophenol production. Equation 1 illustrates the underlying principle of this enzymatic action [25].



2.7.2. Reagents

The alkaline phosphate kit's components included buffer reagent (35 mL) and substrate tablets (10 Nos.).

2.7.3. Working Reagent Preparation

One substrate tablet was dissolved in 3.2 milliliters of buffer reagent to create the working reagent. This working reagent is stored between 2 and 8°C and is stable for at least fifteen days. The substrate is responsive to temperature and light.

2.7.4. Procedure

Two tubes were prepared, one tube for a sample and the second for a blank, and the procedure was carried out in the following steps:

- 1000 µL of working reagent was added to the sample tube.
- The tubes were incubated for one minute at room temperature.
- The sample tubes were filled with 20 µL of serum.
- The blank tube was filled with 1000 µL of distilled water.
- After 30 seconds of thorough mixing, the initial absorbance A was measured.
- Every one to two minutes, the absorbance measurement was taken again.

2.7.5. Calculation

The results were calculated as follows:

$$\text{Activity of ALP (IU/L)} = \frac{\Delta A}{\text{Time}} * 2754$$

2.8. Statistical Analysis

The results were examined utilizing the Statistical Package for the Social Sciences software version 27. This analysis included estimating the mean, standard deviation, confidence interval, and conducting a T-test for comparisons between the two groups. Pearson's correlation coefficient was utilized to assess potential associations between various biochemical indicators.

3. Results and discussion

This study included one hundred participants, fifty of whom had asthma and fifty of whom were controls. The asthmatic and healthy groups did not differ significantly in age. The mean age and standard deviation for the control group were 23.48 ± 1.58 years, while for the asthmatic group, they were 42.00 ± 14.28 years. Significant gender differences were observed; the asthma group had a higher proportion of women than men, which aligns with global trends. Although asthma prevalence is higher in boys during early childhood, the sex ratio varies at puberty, with asthma becoming more prevalent in women. The specific relationship between sex, sex hormones, and the development and persistence of asthma is not yet fully understood. Nevertheless, these elements might contribute to the condition's onset [26]. In contrast, the control group had a larger number of men, likely because they were more willing to participate voluntarily. Gender distribution in the study groups is illustrated in Figure 1.

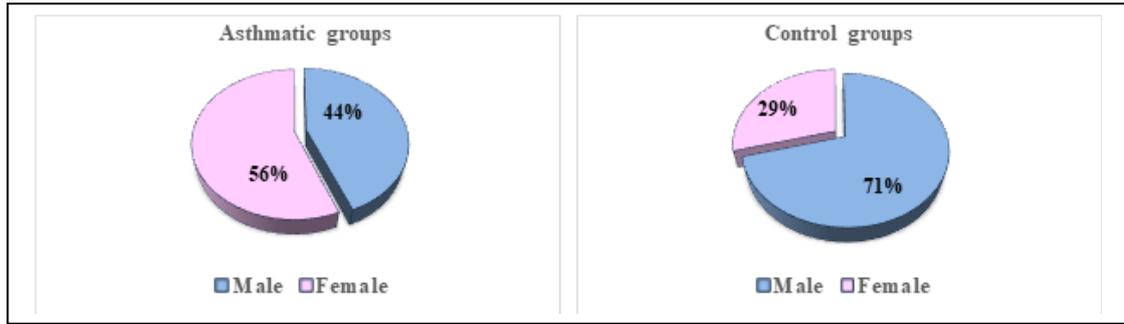


Figure 1 Gender distribution in the study groups

The severity of asthma was estimated only in the patient group, as the control group in this study consisted of healthy group (see Table 2). Most asthma patients in this group reported that they had been diagnosed with asthma since their teenage years, with some having symptoms from a much younger age. The percentage of severity levels observed in our study is higher than the global survey results, which reported a rate of 5% [27,28]. However, consistent with prior reports, the severe category has the lowest proportion of other cases.

In the asthmatic group, 28% of participants lacked formal education, whereas all participants in the healthy group were educated. Additionally, this study demonstrates that the prevalence of asthma varies across urban and rural regions. It is in line with earlier research, as seen in both high- and low-income nations [29,30]. The urban-rural gradient may be caused by a number of variables, such as a Westernized diet, obesity, a sedentary lifestyle, and increased exposure to indoor and outdoor pollution as a result of migration and industrialization [31].

Regarding the relations between asthma and blood group, our result showed that among patients with asthma, the A+ group was the most common blood group by 35%, followed by O+ group by 29%, O- group by 18%, and only 1.6% for AB+ group.

Table 2 Socio-demographic characteristics of the study groups

| Variable | Asthmatic group | Control group |
|--------------|-----------------|---------------|
| Severity | | |
| Mild | 36% | N/A |
| Moderate | 36% | N/A |
| Severe | 28% | N/A |
| Education | | |
| Yes | 72% | 100% |
| No | 28% | 0% |
| Residence | | |
| Urban | 68% | 71% |
| Rural | 32% | 29% |
| Blood Groups | | |
| O- | 18% | 5% |
| O+ | 29% | 63% |
| AB+ | 1.6% | 5% |
| AB- | 0% | 5% |
| A+ | 35% | 11% |
| B+ | 12% | 11% |

This study aims to describe serum ALP activity and body mass index among a population of adults with asthma. Based on BMI values, each study group was classified into two categories: individuals with normal weight (BMI < 30) and those who were overweight or obese (BMI > 30). The study's results indicated significant differences between the patient group and the healthy group, showing that individuals who are obese are more prone to asthma, as shown in Table 3. This finding is consistent with previous research suggesting that obesity has a direct impact on the development of asthma [32].

Table 3 Body mass index in the study groups

| BMI (kg/m ²) | Control | Asthma Patient | Chi-square | P-value |
|--------------------------|---------|----------------|------------|---------|
| Normal weight | 60.00% | 33.33% | 14.21 | 0.0016* |
| Overweight and obese | 40.00% | 66.67% | | |

*P-value less than 0.05 was significant

The results also showed that obese women have a higher percentage than obese men, as seen in Table 4, which agrees with previous studies that women are more susceptible to asthma than men [33].

Table 4 Relation between body mass index and gender

| BMI (kg/m ²) | Male | Female | Chi-square | P-value |
|--------------------------|--------|--------|------------|----------|
| Normal weight | 75% | 25% | 20.53 | 0.00001* |
| Overweight and obese | 43.75% | 56.25% | | |

*P-value less than 0.05 was significant

Table 5 of this study demonstrates that the asthmatic groups' serum alkaline phosphatase levels were considerably greater than those of the healthy controls. No case-control research has looked at the activity of the ALP enzyme in adult asthma to date. Prior studies have mostly focused on children with asthma who were receiving hospital treatment. According to previous research, children with asthma had considerably higher ALP levels than those in the control group and tended to climb over the standard range. The average ALP readings for both groups in this research were within the normal range, although there was a substantial difference between them.

Table 5 Alkaline phosphatase activity in the study groups

| ALP Activity (IU/L) | Mean ± SD | T-test | P-value |
|---------------------|---------------|--------|---------|
| Control | 97.04 ± 24.38 | -2.331 | 0.028* |
| Asthma Patient | 134.94 ± 5.27 | | |

*P-value less than 0.05 was significant

Figure 2 Illustrates the study's findings, which indicate no relationship between ALP activity and BMI

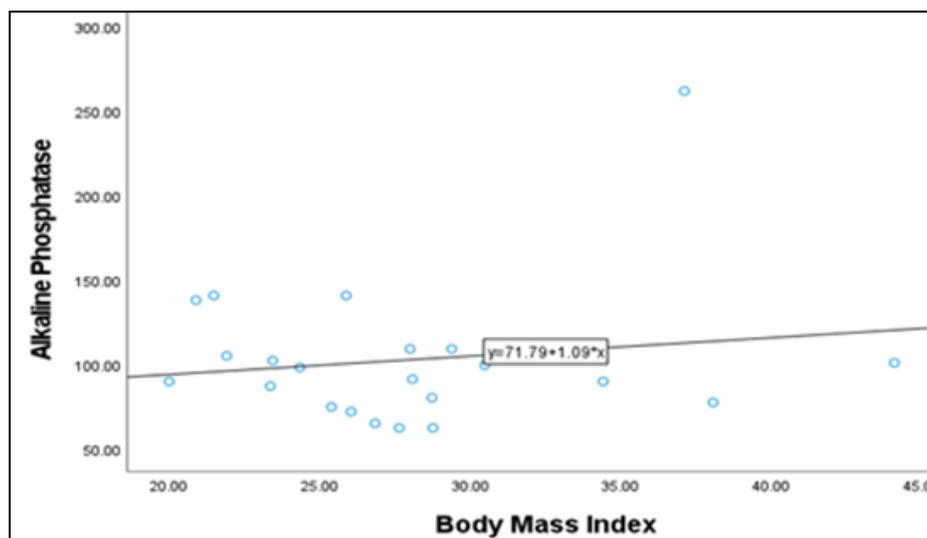


Figure 2 The correlation between alkaline phosphatase and body mass index

4. Conclusion

Significant gender differences were noted, with a higher percentage of women than men in the asthma group. The global survey results, which revealed a rate of 5%, are lower than the severity levels seen in our study. Nonetheless, the severe group had the lowest percentage of other instances, which is in line with earlier findings. Our findings indicate that asthma is more prevalent in individuals with the A+ blood type compared to other blood groups. Furthermore, the Body Mass Index (BMI) data revealed considerably greater inequalities in the asthmatic group compared to the healthy group, suggesting that obese people are more likely to develop asthma. Additionally, the study found that compared to fat males, obese women are more likely to develop asthma. Lastly, serum alkaline phosphatase (ALP) activity was found to be significantly higher in the asthmatic groups than in healthy controls.

Compliance with ethical standards

Disclosure of conflict of interest

There are no conflicts of interest.

Statement of ethical approval

The procedure was approved by the Al-Qasim Green University College of Science's Ethical Committee.

Statement of informed consent

Once the study's goals were explained, all participants gave their written informed consent. Interviews were used to collect baseline clinical and demographic information, which was then recorded using the research questionnaire.

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