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Research Article

Assessment of gluten and lactose intolerance using the cytotoxic test: prevalence and their association

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Abstract-Introduction: Food intolerances—particularly gluten and lactose intolerance—have shown a notable increase in prevalence across Western countries. These conditions can cause a wide range of gastrointestinal and extra-intestinal symptoms, significantly impacting patients' quality of life. Accurate diagnosis is essential to prevent chronic complications and ensure effective dietary management.
Objective: The aim of this study was to assess gluten and lactose intolerance in symptomatic patients using the Cytotoxic Test, and to explore the possible association between these two intolerances based on gender and age distribution.
Materials and Methods: A total of 45 symptomatic patients (23 females and 22 males) were evaluated between March and June 2024. Blood samples were collected and analyzed for leukocyte cytotoxic response to gluten and lactose antigens. Results were classified into four categories: gluten intolerance, lactose intolerance, dual intolerance, and negative.
Results Out of 45 patients, 33.3% tested positive for gluten intolerance, 42.2% for lactose intolerance, and 13.3% for both. Only 11.1% tested negative for either intolerance. Statistical analysis showed a significant association between the two intolerances (OR = 3.125; 95% CI: 1.488–6.563), suggesting that gluten-induced mucosal damage may contribute to reduced lactase activity and secondary lactose intolerance.
Conclusion The Cytotoxic Test appears to be a promising non-invasive diagnostic tool that offers individualized insights into food intolerance. Comprehensive screening for dual intolerance in symptomatic individuals may improve treatment outcomes and patient well-being. Larger-scale studies and methodological standardization are needed to validate these preliminary findings.

Keywords: Food Intolerance, Gluten, Lactose, Cytotoxic Test, Diagnostic Alternatives, Quality Of Life

I. INTRODUCTION

In recent years, there has been a noticeable increase in the number of reported cases of adverse food reactions, particularly in Western countries, encompassing both food intolerances and food allergies (Pereira et al., 2005). In modern diets, these two terms are often confused with each other. However, they are two entirely distinct phenomena.Food allergies are immune-mediated responses that may be either IgE-mediated or non-IgE-mediated (Muraro A, 2014), whereas food intolerance does not result from immune activation but rather manifests with gastrointestinal (Monsbakken K, 2006) and non-gastrointestinal symptoms. These reactions often lead to long-term dietary restrictions or complete avoidance of specific food products, adversely affecting patients' quality of life and frequently resulting in dysbiosis (Onyimba, Crowe, Johnson & Leung, 2021).
Several studies have confirmed the negative impact of food intolerance on quality of life, showing a significant reduction in well-being (Böhn, Störsrud, Törnblom, Bengtsson & Simrén, 2013). Moreover, evidence suggests a potential association between food intolerance and increased anxiety levels (Jansson-Knodell, White, Lockett, Xu & Shin, 2022).
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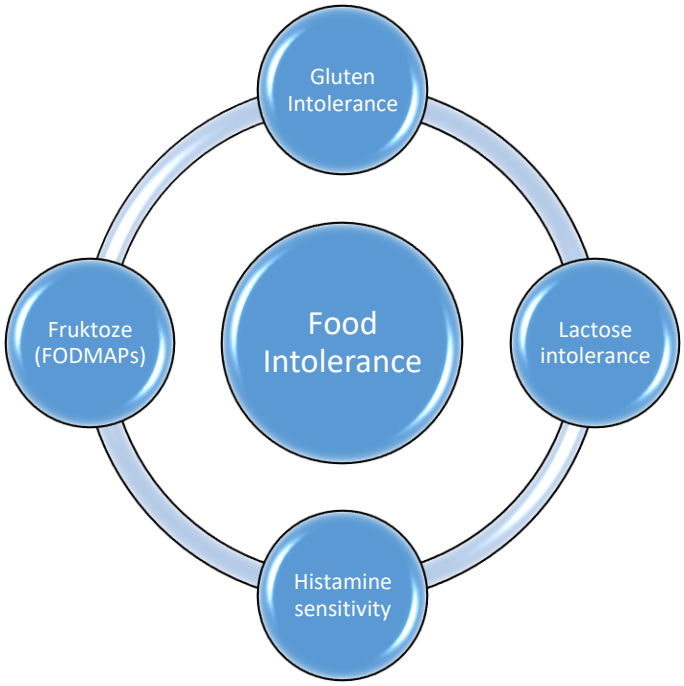

Figure 1. Different types of Food Intolerances.

The figure illustrates the main categories of food intolerances, including enzymatic intolerance (e.g., lactose intolerance), pharmacological intolerance (e.g., histamine sensitivity), intolerance to food additives (e.g., sulfites, MSG), intolerance to fermentable carbohydrates (FODMAPs), and idiopathic or non-specific food sensitivities.Gluten and lactose intolerance are the most common.
Gluten is a complex protein primarily found in cereals such as wheat, barley, and rye, and is a major trigger of several gluten-related disorders, including: **celiac disease**, **gluten allergy**, and **non-celiac gluten sensitivity (NCGS)** (Ludvigsson et al., 2013). The differential diagnosis between these conditions relies on laboratory testing, as intolerance does not involve an immune-mediated response, unlike allergies, but instead involves the production of antibodies directed against the gastrointestinal tract. This is due to the fact that clinical symptoms—whether gastrointestinal or extra-intestinal—are often nonspecific. Some individuals present with such symptoms, but laboratory markers for celiac disease or allergy are absent. In such cases, the condition is referred to as **non-celiac gluten sensitivity** (Cabanillas, 2020). This clinical entity is increasing rapidly and

has a higher prevalence among females, affecting an estimated 0.6–1% of the global population (Roszkowska, Pawlicka, Mroczek, Bałabuszek & Nieradko-Iwanicka, 2019). Risk factors include: family history of celiac disease, food allergies, and autoimmune diseases such as type 1 diabetes and Hashimoto's thyroiditis (Losurdo et al., 2018).

Lactose intolerance is a condition in which the body fails to produce sufficient amounts of lactase, the enzyme necessary for digesting lactose, a sugar found in milk and dairy products. This leads to digestive issues, such as bloating, diarrhea, and abdominal pain after the consumption of lactose-containing foods.

Lactase deficiency may occur in several forms:

- **Primary lactase deficiency** or **lactase non-persistence (LNP)** – a natural genetic trait characterized by a gradual decline in enzymatic activity from childhood to adolescence, with symptoms typically appearing in adulthood.
- **Congenital lactase deficiency** – a rare autosomal recessive condition that manifests from birth (Enattah et al., 2002).
- **Developmental lactase deficiency** – observed in preterm infants (gestational age 28–37 weeks) and is typically transient, resolving with postnatal maturation and breastfeeding.
- **Secondary lactase deficiency** – occurs due to injury to the intestinal epithelium caused by chemotherapy, gastroenteritis, antibiotics, or celiac disease. In such cases, lactase activity usually returns following treatment of the underlying cause (Deng, Misselwitz, Dai & Fox, 2015).

Accurate diagnosis of these intolerances is essential for establishing appropriate treatment and improving the patient's quality of life. Traditional diagnostic methods include serological tests, breath tests, and food tolerance tests, though these methods may have limitations regarding sensitivity and specificity.

In this context, the Cytotoxic Test emerges as an innovative and promising non-invasive method for assessing food intolerance. This test evaluates cellular damage induced by food antigens on the patient's own leukocytes, potentially providing a more direct and individualized insight into cellular reactivity.

II. MATERIALS AND METHOD

Study Objective:

This study aims to assess gluten and lactose intolerance in patients presenting with suspected symptoms, using the Cytotoxic Test, and to perform a comparative analysis of the results based on gender and age groups.

Materials:

A total of 45 patients with suspected gluten and/or lactose intolerance were included in the study. All participants underwent laboratory evaluation during the period March–June 2024. Of these, 23 were female and 22 were male.

Methods:

Blood samples were collected from each patient following standard laboratory protocols, in compliance with ethical guidelines and after obtaining informed consent. The Cytotoxic Test was performed by exposing the patients' leukocytes to gluten and lactose antigens. The level of cellular damage (cytotoxicity**)** was monitored and used as an indicator of food intolerance. The results were categorized as follows:

- Positive for gluten intolerance
- Positive for lactose intolerance
- Positive for both gluten and lactose intolerance
- Negative (no intolerance detected)

III. RESULTS

Table 1. Gender distribution of patients included in the study

Gender	Number of Patients
Female	23
Male	22
Total	45

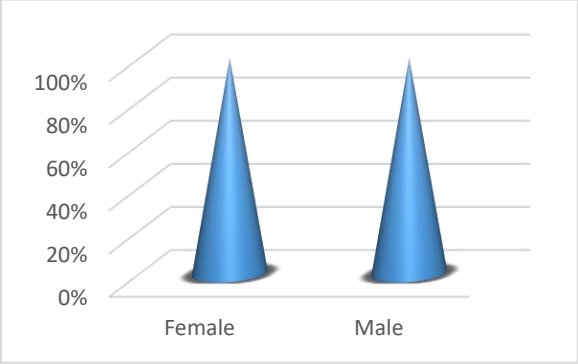

Figure 2. Gender distribution of the subjects in the study

Table 1 and Figure 1 show the gender distribution of the 45 patients included in the study. Females accounted for 23 patients (51.1%),while males accounted for 22 patients (48.9%),indicating an almost equal distribution between the two genders.

Table 2. Laboratory Results of the Cytotoxic Test

Gender	Gluten Positive	Lactose Positive	Gluten and Lactose Positive	Negative Intolerance	Total
Female	10	8	2	3	23
Male	5	11	4	2	22
Total	**15**	**19**	**6**	**5**	**45**

Out of the 45 patients included in the study,15 (33.3%) tested positive for gluten intolerance, 19 (42.2%) were positive for lactose intolerance, and 6 (13.3%) tested positive for both gluten and lactose intolerance. Only 5 patients (11.1%) tested negative for any food intolerance.

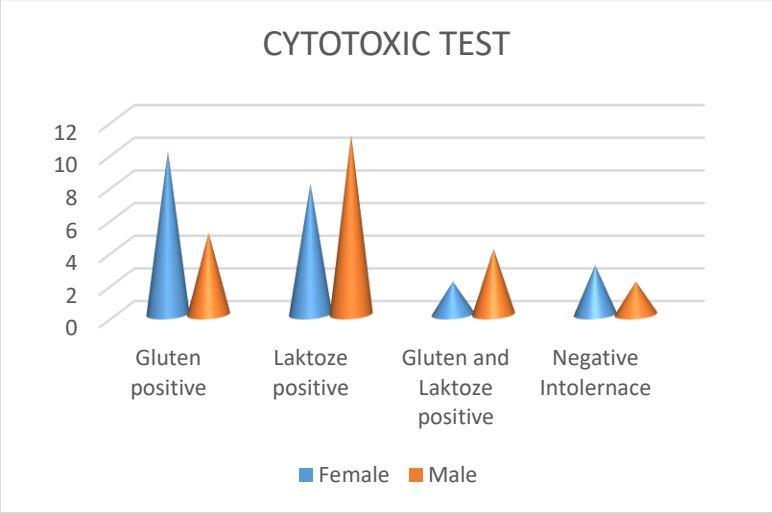

Figure 3 Distribution of results by gender

Gender-based analysis showed that females had a higher prevalence of gluten intolerance (10 females) compared to males (5 males), whereas lactose intolerance was more frequent among males (11 males) than females (8 females). Combined intolerance (both gluten and lactose) was also more common in males (4 males) than in females (2 females).

Table 3. Odds Ratios (OR) for Lactose Intolerance Based on Gluten Intolerance Status

Variable	Odds Ratio (OR)	95% Confidence Interval (CI)
Odds Ratio for Lactose Intolerance (1/2)	0.105	0.027 – 0.413
Gluten Intolerance Group = 1	0.329	0.149 – 0.725
Gluten Intolerance Group = 2	3.125	1.488 – 6.563

This study evaluated the association between lactose intolerance and gluten intolerance in a sample of 45 patients.The results showed that patients with gluten intolerance (coded as Group 2, i.e., positive) had a 3.13-fold increased likelihood of also having lactose intolerance compared to the reference group (OR = 3.125; 95% CI: 1.488–6.563). This odds ratio is statistically significant, as the confidence interval does not include the value 1.Conversely, patients classified in Group 1 for gluten intolerance demonstrated a reduced probability of lactose intolerance (OR = 0.329; 95% CI: 0.149–0.725).The OR value of 0.105 (95% CI:

0.027–0.413) for the lactose intolerance comparison (1/2) confirms a significant difference in the presence of lactose intolerance across different gluten intolerance groups. These findings suggest a strong association between gluten and lactose intolerance, underscoring the importance of evaluating both conditions in clinical practice to ensure accurate diagnosis and appropriate dietary management.

IV. DISCUSSION

This study demonstrates a considerable prevalence of gluten and lactose intolerance among the evaluated patients, as determined by the cytotoxic test. The findings indicate that lactose intolerance was more frequent than gluten intolerance, aligning with existing literature that highlights lactose intolerance as a common condition. Gender-based distribution showed that females were more frequently affected by gluten intolerance, while males had a higher incidence of lactose intolerance and combined intolerance. Our results also indicate a strong and statistically significant association between gluten and lactose intolerance. Patients who tested positive for gluten intolerance (coded group 2) were approximately 3.1 times more likely to also develop lactose intolerance. This finding is consistent with previous studies suggesting that mucosal damage caused by gluten reactivity may impair the intestinal capacity to digest lactose. Chronic inflammation and villous atrophy, commonly seen in celiac disease or gluten-related disorders, may reduce lactase enzyme activity, leading to secondary lactose intolerance.

Conversely, patients coded as group 1 gluten intolerance (possibly negative or mild symptoms) showed a lower probability of lactose intolerance, indicating that the extent of mucosal damage or inflammatory activity may significantly influence the manifestation of dual intolerance.These results emphasize the importance of comprehensive diagnostic evaluation in patients with gluten intolerance, including assessment for lactose intolerance, to define a more effective dietary management strategy and improve patient quality of life.A key strength of this study is the use of the Cytotoxic Test as a diagnostic tool. This test emerges as a promising alternative to traditional serological tests. Unlike antibody-based assays, the cytotoxic test directly assesses the cellular response to food antigens, providing a more immediate and individualized snapshot of intolerance. This method is non-invasive, capable of detecting intolerances not clearly identified by antibody levels, thus expanding diagnostic potential. The advantages of the cytotoxic test include rapid results, repeatability for monitoring, and increased patient compliance, especially for those seeking safer and less invasive alternatives to elimination diets or challenge-based testing.However, the cytotoxic test also presents limitations. One major challenge is the lack of full standardization across laboratories, and the need for further research to validate its accuracy and specificity compared to established diagnostic tools. Additionally, the relatively small sample size in our study limits statistical power and may affect the generalizability of the findings.The implementation of cytotoxic testing could enhance early diagnosis and management of food intolerances in clinical practice, potentially reducing complications and improving patients' quality of life.In conclusion, the cytotoxic test represents a valuable complementary and alternative tool to serological assays, with the potential to improve clinical practice in the evaluation of gluten and lactose intolerance. Further studies with larger sample sizes and standardized laboratory protocols are needed to confirm and expand these findings.

Clinical Practice Recommendations:

- Lactose intolerance screening should be considered in patients diagnosed with gluten intolerance as part of standard evaluation protocols.
- Patient education regarding the possibility of dual intolerance and combined dietary management may improve treatment outcomes and symptom reduction.
- Further research is needed to explore the molecular mechanisms linking these two intolerances and to develop targeted therapeutic strategies.

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