

# Assessment of GATA3 expression in duodenal biopsies of celiac disease suspected and diagnosed patients

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**Objective** In this study we aimed to assess the staining of GATA3 in the duodenal biopsy of celiac disease (CD) patients as step toward improving its diagnosis.

**Methods** Duodenal biopsied tissues from 50 patients suspected of celiac disease were examined by conventional staining followed by immunohistochemistry using monoclonal antibody GATA3 for tissue sections.

**Results** The histopathological examination of the samples showed a low GATA3 staining in the duodenal tissue of CD patients.

**Conclusion** This study concludes that the low staining of GATA3 in the duodenal CD patients indicates the little consequence of Th2 lymphocyte in the disease pathogenesis. This finding is of importance and could be of use in the diagnosis of celiac disease.

**Keywords** GATA3, celiac disease, gluten-sensitive enteropathy, immunohistochemistry

## Introduction

Celiac disease is also known as gluten-sensitive enteropathy.<sup>1</sup> It is a chronic, immune-mediated disease occurring in genetically predisposed individuals<sup>2</sup> due to an intolerance to gluten-containing foods.<sup>3</sup> The architectural changes in the mucosal villi are a result of an abnormal immune response caused by this intolerance.<sup>3,4</sup>

Celiac disease is increasing in prevalence. It is currently estimated at 1:100 in Western countries,<sup>5</sup> and 1.5:100 in the Middle Eastern populations,<sup>6–8</sup> CD may occur in adults and children. The female/male ratio of CD is 3:1.

The diagnosis depends on clinical, serological and pathological indications. Serology is the first line of the tests performed. Testing for antibodies for tissue transglutaminase (tTG), deaminated gliadin peptides (DGP) of IgA and/or IgG. Nevertheless, biopsy is still the gold standard for confirmation of the diagnosis.<sup>9–11</sup>

The intraepithelial lymphocytes (IELs) comprise of a significant T-lymphocyte population.<sup>12–15</sup> Residing adjacent to the lumen of the intestine, between enterocytes in the intestinal epithelium and act as sentinels protecting the epithelial barrier.<sup>16</sup> In healthy individuals IELs are of approximately 75% are CD8+ T cells and 10% are CD4+ T-cells and 15% are either double negative or CD8+.<sup>17</sup> B cells are not present. The immunological functions of IELs are cytotoxic action and cytokines secretion.<sup>18,19</sup> Their count does not exceed 25 IELs/100EC in normal mucosa.

GATA3 is a transcription factor. It is regulator of both innate and adaptive immunity.<sup>20</sup> GATA3 has a critical importance in the development and function of immune cells like T cell, B cell and natural killer (NK).<sup>21–26</sup>

Extensive studied have investigated GATA3 function in T cells (Zhang and Bevan, 2011). It has been originally identified to be a main regulator for Th2 differentiation of CD4+ T cells. Yet there is mounting proof proposing the crucial role of GATA3 in development, differentiation, and function of other CD4+ T cell subsets, as well as CD8+ cells.<sup>27–29</sup>

In this article, we will focus on assessing the staining of GATA3 transcription factor in the tissue samples of CD suspected and diagnosed patients.

## Materials and Methods

The samples of 50 consecutive patients were collected. Twenty samples (40%) were of CD diagnosed patients. While the other 30 (60%) were of inconclusive histopathology. All samples were formalin fixed paraffin imbedded tissue. They were collected from Al-Hussainy Hospital, Al Sajjad Medical Laboratory and Al-Kafeel Hospital in Karbala City from August 2017 until February 2018. Processing of the samples and staining with H&E was performed. Then immunohistochemistry was performed using the GATA3 mouse monoclonal antibody, Santa Cruz Biotechnology, Inc. to track the Th2 lineage. A detection kit was purchased from Leica Biosystems Newcastle Ltd., UK, Novolink™ Polymer Detection System. Data were analyzed using IBM SPSS analytic software.

## Results

The age of patients was ranged from 10 to 60 years. The female to male ratio was 3:1. The patients were diagnosed based on a combination of clinicopathological indications. The 20 (40%) diagnosed samples had a positive serology and an indicative histology of CD represented by increase of IELs >30/100EC, crypt hyperplasia and architectural changes of the intestinal villi. While the other 30 (60%) cases had no architectural changes with only increase of IELs >25 IEL/100EC. All samples were stained with GATA3 monoclonal antibody. The GATA3 monoclonal antibody was used to trace the Th2 lineage of lymphocytes in the collected tissue samples. The cells labeled by GATA3 in the duodenal tissue showed a brown nuclear color indicated by the DAB chromogen. While other cells stained with the hematoxylin blue. The stained IELs were counted in each sample. A percentage was extracted of cells stained with GATA 3 from the total number of IELs counted by H&E. The count of IELs stained by the GATA3 monoclonal antibody was very low in number. The microscopic image of the GATA3 stained cells was very distinct by their contrast. The resulting counted percentage of the stained cells by GATA3 were 2–5% of the total cells count by H&E. Low count of IELs was seen in

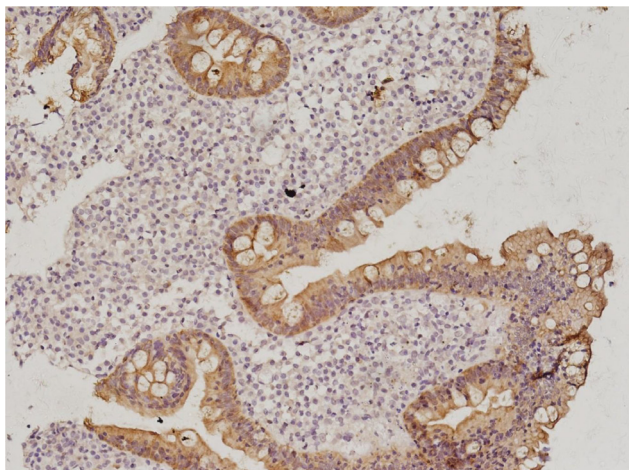


Fig. 1 **Staining of GATA3 in duodenal biopsies of celiac disease diagnosed patients.**

all cases, Figure 1. A very notable phenomenon was the background staining of the GATA3 of the duodenal tissue. This observed staining could be of the epithelial cells of the villi.

A very notable phenomena was the background staining of the GATA3 of the duodenal tissue mostly concentrated in the lamina propria.

## Discussion

The GATA3 is a zinc-finger transcription factor highly expressed in naïve, freshly activated Th2 lineage.<sup>30</sup> It has been proposed that celiac disease is of a Th1 mediated immune response.<sup>31–35</sup> Few data are available on the role of Th2 lineage in duodenal tissue samples. Although GATA3 have been used as a tumors marker for studying and diagnosing many tumors

including but not limited to breast and urothelial carcinomas.<sup>36</sup> With the exception of salivary gland and parathyroid tumors, GATA3 has been reported to be either absent or only rarely expressed in other epithelial tumors.<sup>37</sup> A recent study shows that GATA3 was negative in the normal duodenum tissue,<sup>36</sup> meaning the absence of Th2 in that tissue. In this study, we used the GATA3 to test the hypothesis that celiac disease is of Th1 mediated immune response by searching for the Th2 lineage in celiac disease diagnosed cases. We found that GATA3 (Th2) staining in the cells was in about <5%. Indicating the weak presence of Th2 in the tissue. This further strengthens the suggestion that celiac disease is of Th1 mediated response. In general the GATA3 showed a non-specific staining. These results are similar to another study by Jöhrens et al.<sup>30</sup> where they also studied the role of GATA3 in celiac disease samples. There results were consistent with what we found, GATA3 expression in the duodenum samples of celiac disease patients was very low in whether it was in the villi or in the lamina propria. These data serve as evidence supporting the significant role of the Th1 immune cells in celiac disease.

## Conclusion

GATA3 staining in the duodenal tissue of celiac disease patients is very low. As was with the normal duodenum patients with no histopathological indications. This could be helpful in the diagnosis of CD.

## Conflicts of Interest

None declared.

## Financial Disclosure

None declared. ■

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