WHITE PAPER

VIBRANT WELLNESS

WHEAT ZOOMER - RA OVERLAP

Table of Contents

1. Rheumatoid Arthritis (RA)
2. Wheat Sensitivity (WS)
3. Lack of Investigation on Rheumatoid Arthritis Characteristic Markers in Wheat Sensitivity
4. Our Study: High Frequency of Rheumatoid Arthritis Markers in Wheat Sensitivity
5. Conclusion
6. References

1. Rheumatoid Arthritis (RA)

Rheumatoid arthritis (RA) is a systemic autoimmune disease that causes progressive articular damage, functional loss, and comorbidity. RA manifests as pain, stiffness, swelling, and functional impairment in joints and is characterized by persistent synovitis, chronic inflammation, and the presence of specific autoantibodies. As symptoms of RA can closely mirror other diseases, it is usually difficult to diagnose in the early stages. One of the most important and helpful criteria in reaching a RA diagnosis is to identify antibodies to rheumatoid factor (anti-RF) IgM and anti-cyclic citrullinated peptide (anti-CCP) IgG and IgA, as shown in Table 1. Despite unknown etiology, new interest has emerged in studying the interaction of RA with other diseases due to the opportunity for the development of novel diagnostic and therapeutic strategies for patients with comorbidity.

Table 1. Rheumatoid Arthritis Panel

Marker	Sensitivity	Specificity
Anti-cyclic citrullinated peptide (CCP) IgG and IgA[1]	~80%	~98%
Anti-rheumatoid factor (RF) IgM	~70%	~90%

2. Wheat Sensitivity (WS)

Wheat-related disorders occur in a broad spectrum of conditions through the ingestion of wheat and its major storage protein, gluten. Individuals usually develop gastrointestinal symptoms such as abdominal pain, diarrhea, and bloating. Celiac disease (CD), as the most common wheat-related disorder, is an autoimmune disorder precipitated in genetically predisposed individuals and it affects about 1% of adults and children in the United States.[2]

In addition to CD, we are currently witnessing a significant proportion of individuals encountering gastrointestinal and extra-intestinal symptoms related to wheat ingestion in the absence of CD or wheat allergy. Wheat Zoomer (WZ), which detects IgG and IgA Antibodies to 18 wheat proteins at the peptide level listed in Table 2, is an extremely useful tool in assessing the possible causes for such individuals.[3] All the key proteins of wheat are arrayed on the Vibrant Wheat Zoomer chip as overlapping 18-mer peptides covering the entire protein.

Table 2. Protein probes of Wheat Zoomer (WZ)

PANEL	PROTEINS
Celiac Disease (CD)[3]	Transglutaminase 2 (tTG), deamidated gliadin peptide (DGP)
Transglutaminase[4,5]	Transglutaminase 3, Transglutaminase 6
Wheat Germ[6]	Wheat Germ Agglutinin
Gliadin[7]	α Gliadin, α-β Gliadin, γ-Gliadin, Ω Gliadin, Gluteomorphin, Prodynorphin
Glutenin[8]	Low molecular weight glutenin, High molecular weight glutenin
Non-gluten Protein[9]	Serpin, Farnins, Amylase/Protease Inhibitors, Globulins, Purinin

3. Lack of Investigation on Rheumatoid Arthritis Characteristic Markers in Wheat Sensitivity

Despite being separate disease states, RA and CD share multiple aspects in epidemiology and clinical manifestations. The epidemiology of both disorders has been proven to be influenced by comparable environmental factors and recent incidental surge of associated antibodies. The infectious, dysbiotic and increased intestinal permeability theories, as drivers of autoimmune cascade, apply to both CD and RA. Although RA and CD differs in HLA pre-dispositions and specific predictive and diagnostic biomarkers, the pathophysiology of both diseases are both mediated by endogenous enzymes target at different organs. There are extensive studies evaluating presence of CD characteristic biomarkers in RA patients while studies on RA characteristic biomarkers in CD patients are very rare. Lubrano et al. demonstrated a 26% prevalence of arthritis among 200 adult CD patients.[5] In another study of determining the frequency of celiac markers in 85 RA patients, anti-gliadin IgG antibodies were positive in 16 patients, anti-gliadin IgA in 29 patients, ultra-purified antigliadin in 14 patients, and only one patient had anti-tTG.[10]

4. Our Study: High Frequency of Rheumatoid Arthritis Markers in Wheat Sensitivity

In this study, we investigated the overlap of characteristic biomarkers in Rheumatoid Arthritis, Celiac Disease, and Wheat Sensitivity. The aim of our study was to assess the presence of RA markers in subjects with wheat-related disorders and the presence of wheat protein antibodies (gluten and non-gluten proteins) in RA subjects. Understanding the biomarker overlap not only provides significant diagnostic importance, but also may ultimately contribute to the prompt treatment decisions.

Methods

A total of 844 subjects with joint pain and/or gastrointestinal symptoms related to wheat ingestion were addressed to the Vibrant America Clinical Laboratory from November 2015 to January 2018. Serum samples were collected from all subjects and tested with the CD panel, the WZ panel, and the RA panel including anti-cyclic citrullinated peptide and anti-rheumatoid factors IgM. Retrospective analysis was completed using de-identified clinical data and test results.

Results

(A) Prevalence of RA markers in CD and WS individuals

The prevalence of RA markers, anti-RF IgM and anti-CCP3 IgG and IgM, were investigated in the CD+ subjects, WZ+ subjects, and the NWS controls, as shown in Figure 1. Among 49 CD+ subjects, RA markers were found in 10 (20%) subjects. Of the 10 subjects who carry both CD and RA markers, 5 (10%) subjects had anti-CCP3 IgG/IgM and 5 (10%) subjects had anti-RF IgM. Interestingly, the presences of the anti-CCP3 IgG/IgM and anti-RF IgM were complementary and there were no subject carrying both at the same time. Among 605 WZ+ subjects, RA markers were detected in 106 (18%) subjects.

Among these 106 subjects, 76 (12.5%) were seropositive to anti-CCP3 IgG/IgM and 37 (6.1%) were seropositive to anti-RF IgM, meaning 7 of the WZ+ subjects carried anti-CCP3 IgG/IgM and anti-RF IgM simultaneously. The control group consisted of 190 NWS subjects who were seronegative in both CD and WZ panels. 12 (6.3%) of the NWS subjects were seropositive in the RA panel. Among them, 8 (4.2%) were positive to anti-CCP while 5 (2.6%) were positive to anti-RF IgM, meaning only one NWS subject had both positive anti-CCP3 IgG/IgM and anti-RF IgM.

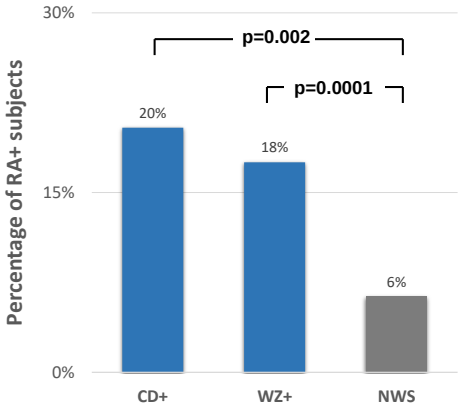

Fig 1. High frequency of RA markers in CD and WZ seropositive subjects ($p<0.05$ is considered as significantly different).

Results

(B) Prevalence of CD and WZ markers in RA individuals

The prevalence of CD and WZ markers were intensively researched in the RA seropositive subjects and seronegative symptomatic controls, as shown in Figure 2. In 128 RA+ subjects, 10 (7.8%) were found to have one or more CD hallmark antibodies (anti-tTG IgG, anti-tTG IgA, anti-DGP IgG, anti-DGP IgA). Anti-tTG2 IgG (9/128, 7%) were the most frequently found CD marker, followed by anti-tTG2 IgA (2/128, 1.6%) and anti-DGP IgA (1/128, 0.8%). All RA+ subjects in this cohort showed negative to anti-DGP IgG.

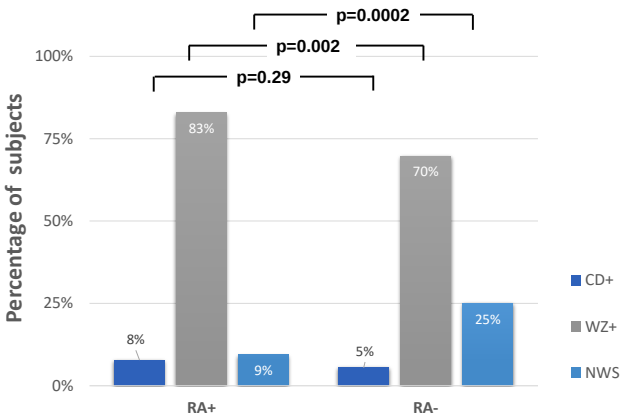

Fig 2. Prevalence of CD and WZ markers in RA positive and negative subjects ($p<0.05$ is considered as significantly different).

The frequencies of Wheat Zoomer markers, which are clinically relevant antibodies to significant wheat-related disorders, were also investigated within the same 128 RA+ subjects. 106 (83%) were found to be seropositive for at least one marker in the Wheat Zoomer panel while being seronegative in the CD panel. Moreover, 12 (9%) NWS subjects were found in the RA+ subjects. The prevalence of the same sets of subjects were also investigated in the 716 RA- controls. 39 (5%) CD+ subjects, 499 (70%) WZ+ subjects, and 178 (25%) NWS subjects were found in these symptomatic seronegative controls.

Results

(C) Frequencies of WZ markers in the RA+ subjects

Among the 128 RA+ subjects, anti-gliadin (95, 74%) were the most frequently detected WZ antibodies, followed by anti-non-gluten wheat protein antibodies (57, 44%), anti-wheat germ (57, 44%), and anti-glutenin (47, 37%). In 716 RA- controls, anti-gliadin (467, 65%) were also the most frequently detected WZ antibodies, followed by anti-non-gluten wheat protein antibodies (332, 46%), anti-wheat germ (332, 46%), and anti-glutenin (234, 33%).

4. Conclusion

In conclusion, our data presents a strong association of the characteristic serological biomarkers between rheumatoid arthritis and wheat-related disorders. From the point of view of a clinical laboratory, we observed a significantly greater frequency of RA markers in seropositive subjects with wheat-related disorders as well as wheat protein antibodies in RA subjects compared with respective seronegative controls. We believe this result is worthy to be explored in more general populations and to be considered while conducting prognosis for both conditions. Accessing disease biomarkers at an early stage will potentially help in lifestyle choices to ameliorate symptoms. Owing to the development of high-throughput quantitative antibody-based assays, pursuing multiple panel testing at an early stage becomes economically possible and could provide enormous potential in improving diagnosis accuracy and predicting disease development.

5. References

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