The Relevance of Using the C3d/IgG Test in Clinical Intervention

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Abstract

Background: A large subset of the population is afflicted with a wide range of food-related inflammatory conditions, with at least 100 million people affected worldwide.

Primary Study Objective: To validate the food sensitivity C3d/IgG test for its ability to manage patients with intestinal and extraintestinal symptoms.

Methods: The study was a retrospective analysis of 30 subjects ranging from age 7-71, consisting of 9 males and 21 females. Outcomes are based upon the status of primary complaints after being placed on an exclusion dietary regimen based on elevated serum C3d/IgG food-specific antibodies. Two C3d/IgG tests were performed on the patient’s serum by the method of Indirect Enzyme Linked Immunosorbent Assay (ELISA). From the initial test, elevated anti C3d/IgG foods were identified and eliminated from patient’s diet. Subjects were retested at an average of 10.7 months; both food sensitivities and chief complaints were reassessed.

Intervention: The C3d/IgG test measures both the innate and adaptive responses of the immune system. The test quantifies IgG antibodies and the inflammatory biomarker, C3d, with magnitude of reaction on a scale of severe, high, moderate and mild. Food reactions with the exception of mild were eliminated from the individual’s diet. Then subjects were retested to determine if their symptoms improve with food elimination.

Results: Patients who complied with the avoidance of anti-C3d/IgG dietary antigens demonstrated statistically significant reduction in C3d/IgG testing sensitivity, and marked reduction in symptoms that they reported before beginning the diet. The p-values are 1.56E-06, 0.007, and 0.001 for the severe, high, and moderate test results between the initial and second test.

Conclusion: Overall, patient well-being improved when C3d/IgG food sensitivity decreased as a result of an exclusion diet, demonstrating food removal based on the C3d/IgG test is an effective approach in patient care.

Keywords: Delayed Hypersensitivity reaction, food sensitivity, zonulin, intestinal permeability, immunoglobulin (Ig), complement, inflammation, antigen
Introduction

A great amount of time has been invested in studying the mechanism and effector molecules involved in food allergies. Effective pharmacotherapies have been formulated to alleviate a wide variety of patients’ symptoms, but nothing close to being curative. Food allergies involve two major types of reactivity, immediate (IgE-mediated) and delayed (IgG-mediated). IgE mediates Type 1, and IgG mediates Type II/III hypersensitivity reactions. Immunological Food Sensitivities are characterized as hypersensitivity and inflammatory responses to an immune-mediated reaction from the ingestion of an offending food.²

Figure 1. Classification of Food Sensitivity. Figure was obtained with permission from authors of Food Allergies and other Food Sensitivities, which was initially adapted from the Institute of Food Technologist, 1985.²
The inflammatory response is a recurring theme to all allergic reactions resulting in release of various effector molecules namely; histamine, serotonin, tumor necrosis factor and arachidonic acid metabolites. Subsequently, vasodilation, increased vascular permeability, edema, smooth muscle contraction, chemotaxis and tissue damage are reactions to the effects of these chemical mediators. Immediate and delayed hypersensitivity reactions differ in that, IgE is acute, whereas IgG is insidious, hence, their names immediate and delayed, respectively. The table below list differences classically observed in both types of food allergies.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Immediate Food Allergy</th>
<th>Delayed Food Allergy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunoglobulin (Ig)</td>
<td>IgE-Mediated</td>
<td>IgG-Mediated</td>
</tr>
<tr>
<td>Ig half-life</td>
<td>1-2 days</td>
<td>21 days</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>Tend to be permanent</td>
<td>Diminishes by avoidance</td>
</tr>
<tr>
<td>Onset of Symptoms</td>
<td>Immediate phase: ≤ 8 hours</td>
<td>Delayed: ≥24 hours</td>
</tr>
<tr>
<td>Duration of symptoms</td>
<td>Acute</td>
<td>Chronic</td>
</tr>
<tr>
<td>Amount of Exposure required for Immunoreaction</td>
<td>Infrequent exposure</td>
<td>Frequent exposure</td>
</tr>
<tr>
<td>Mortality and Morbidity</td>
<td>High probability of fatality (i.e. anaphylactic shock; low morbidity)</td>
<td>Low fatality, High morbidity</td>
</tr>
<tr>
<td>Dose</td>
<td>Not quantitative (all or none reaction)</td>
<td>Dose dependent</td>
</tr>
<tr>
<td>Mechanism</td>
<td>Basophil/Mast Cell</td>
<td>Classical Complement Pathway</td>
</tr>
<tr>
<td>Chemical Mediators</td>
<td>Histamine, Leukotriene</td>
<td>Immune Complex, Helper T cells, Cytokines</td>
</tr>
<tr>
<td>Effector Function</td>
<td>Vascular Permeability, Smooth Muscle Contraction</td>
<td>Inflammation, Tissue damage</td>
</tr>
</tbody>
</table>

Table 1. Differences between Immediate and Delayed Food Allergies.

Acute, IgE, hypersensitivity reaction has been well studied and is highlighted by the most serious complication, anaphylactic shock. Delayed, IgG, reaction equally demands attention considering the wide range of symptoms it causes. The remainder of this paper will be focused on IgG, and C3d, a biomarker of the complement pathway system, and their significance in clinical intervention in managing patients with debilitating symptoms.

The pathogenesis of delayed food allergies is facilitated by intestinal permeability that results in a compromised gastrointestinal lining contributing to the loss of tolerance and provocation of the immune system. As an example, gluten, found in wheat, increases intestinal permeability and IgG, elevates complement, and has a temporal relationship
with autoimmune disease, particularly, Celiac disease. Among other foods, casein and
whole milk are reported to contribute to the elevation of IgG antibodies in children with
autistic spectrum disorders (ASD). When children with ASD were monitored using a
two-stage, randomized, controlled study, and placed on a gluten free casein free diet,
researchers reported dramatic improvements in behavioral pattern after 8 months.

Within the last decade much effort has been directed in an attempt to elucidate the
correlation between intestinal permeability and autoimmune diseases such as Celiac
disease. Recent studies suggest an interplay between environmental factors and genetic
susceptibility being integral components in the establishment of certain autoimmune
diseases. Celiac disease (CD) is characterized by chronic inflammation of the small
intestine caused by ingestion of gluten, resulting in the destruction of villi, and
subsequently malabsorption. These individuals have either one or both classes of major
histocompatibility complex (MHC) II haplotypes, HLA DQ2 or HLA DQ8. Since the
discovery of zonulin, the unilateral concept of molecular mimicry being the cause of
autoimmune pathologies has given way to the emergence of a new paradigm, intestinal
permeability. The C3d/IgG test is validated in the management of patients with various
symptoms and intestinal permeability, which is an integral component of many disease
processes, and may prove to be useful in the zonulin-related diseases listed below.

- Autoimmune: Ankylosing Spondylitis, Celiac Disease, Crohn’s Disease, Rheumatoid Arthritis, Systemic Lupus Erythematosus, Type I Diabetes Mellitus
- Cancer: Glioma, Breast, Lung (adenocarcinoma), Ovarian, Pancreatic
- Neurological: Autism, Multiple Sclerosis, Schizophrenia
- Infection: Sepsis
- Metabolism: Obesity

Zonulin, a marker of intestinal permeability, is associated with obesity-induced insulin
resistance metabolic disturbances, and elevations of interleukin-6 (IL-6), a cytokine of
diverse functions. In B-lymphocytes it promotes terminal differentiation, in plasma cells
it promotes antibody secretion, and in hepatocytes it induces the synthesis of acute phase
proteins, such as the complement component, C3. Another study documented by
Klaus et al shows a correlation between zonulin and sepsis. Combined these diseases
attest to the strong inflammatory component, measurable using the C3d/IgG tests,
incurred by intestinal permeability and its multiple organ involvement, owing to C3d
being a robust indication of inflammation. Zonulin, the human analog of zonula occluden
toxin (Zot), an enterotoxin from Vibrio cholerae that reversibly alters the intercellular
tight junction permeability, has shed light on trafficking of macromolecules along a
paracellular pathway, influencing the balance that exist between immune activation and
tolerance. When zonulin is upregulated through food antigens, particularly, gluten, there
is an increase in gut permeability, which sets the stage for inflammation driven by the
increase in IgG and complement. Zonulin causes the disassembly of tight junction,
resulting in loss of the intestinal barrier integrity. It is known that gluten, a
proteinaceous antigen, consisting of glutenins and gliadins, induces intestinal
permeability. This occurs when gliadin binds chemokine receptor CXCR3, which results
in the activation of myeloid differentiation primary response 88 (MyD88), promoting the
epithelial release of zonulin, only when gliadin is exposed to the apical surface of the
intestinal wall\textsuperscript{15,27,28} As a consequence of intestinal permeability, antigens that would otherwise be isolated to the lumen of the gastrointestinal tract are now afforded access to the submucosa and ultimately, the blood stream resulting in immune activation. Chronic inflammation is associated with asthma, chronic fatigue syndrome, depression, inflammatory bowel disease and irritable bowel syndrome and inexplicable symptoms that are mentioned by individuals.\textsuperscript{1,29-31} Zonulin provides us with evidence that tight junctions are regulated in a dynamic process, influencing physiological, developmental and pathological activities.\textsuperscript{9,32} Proteins from foods will pass through tight junctions via a paracellular route, upregulating the immune response, thereby evoking an inflammatory reaction and creating varied symptoms of general food sensitivity. When regulation has been lost and symptoms ensue, the most effective approach is elimination of the dietary antigens resulting in upregulation of complement and immunoglobulins. The removal of foods decreases the antigenic load and neutralizes the inflammatory response. Tight junctions will close, the gastrointestinal integrity of the mucosa is reestablished, and the host’s immune competence are restored.\textsuperscript{10}

In addition to the gastrointestinal system digestive and absorptive functions, its unique anatomical and functional arrangements contribute to its ability to perform motility, neuroendocrine and immunological functions.\textsuperscript{33} The gastrointestinal tract boasts a large intestinal mucosal surface, fortified with an impressive immune system, notably, the gut-associated lymphoid tissue (GALT), with approximately a trillion lymphoid cells per meter of the small intestine.\textsuperscript{34} Intraepithelial lymphocytes are positioned between epithelial cells, and a predominance of B-lymphocytes in Peyer’s patches located in the lamina propria.\textsuperscript{10} The immunological function is of significant interest, due to its involvement with inflammatory reaction, may cause intestinal and extraintestinal symptoms triggered by food antigens: bloating, abdominal pain, diarrhea, constipation, fatigue, migraines, headaches cognitive dysfunction, depression, myalgia, joint pain, sinusitis, and urticaria, are a few of the symptoms described by patients.

By measuring IgG and C3d, delayed food allergy reaction (also referred to as non-IgE cell mediated reaction) is identified, quantifies the degree of permeability and directs the clinical intervention. The two facets of the immune system are measured: innate and adaptive, corresponding with C3d and IgG, respectively. C3d, a cleavage fragment of the complement cascade pathway is measured, in conjunction with immunoglobulin G (IgG) 1-4 subtypes to quantitate the severity of individuals’ inflammatory response to various offending foods. In chronic disease states, for example hepatitis and rheumatoid arthritis IgG levels are markedly high. Serum IgG levels, also elevate when food antigens permeate the intestinal wall at an abnormally high rate.\textsuperscript{10} C3d is also elevated in patients with Systemic Lupus Erythematosus, Rheumatoid arthritis, Membranoproliferative Glomerulonephritis and Hepatic cirrhosis from alcohol misuse.\textsuperscript{35-39} As a consequence, increased serum IgG levels to multiple foods implicates intestinal permeability. Moreover, this perpetuates a vicious cycle, as increased permeability, puts a greater antigenic burden on the immune system, which in turn stimulates a hypersensitivity reaction to food antigens and components of the gut flora. Inflammation continues, further adding injury to insult on the intestinal barrier sustaining the destruction.\textsuperscript{10,40,41}
The use of C3d as a quantifiable parameter in the C3d/IgG test enhances the accuracy, and is a reliable biomarker of tissue inflammation linking the innate and adaptive immune response.\textsuperscript{42-44} It amplifies the sensitivity and improves the reproducibility of the test. C3d is a proteolytic fragment of C3dg that has the ability to function as molecular adjuvant to augment humoral immune responses. In 2000, Ross et al. demonstrated the ability of C3d to enhance antibody production to establish immunity against an attenuated virus.\textsuperscript{45} Hass et al. demonstrated that C3d exerts its adjuvant-like activities by targeting antigens to C3d receptor (CD21/35), which interacts with CD19 to regulate B cell activation increasing antibody production.\textsuperscript{46} The following figure depicts how food antigens enter the submucosa of the gastrointestinal tract via paracellular passage, influenced by zonulin to incite an inflammatory process producing various symptoms from deposition of immune complexes in tissue of organs. The conductor of this inflammatory process is the CD4\textsuperscript{+} helper T cells, particularly; type 1 response, characterized by the production of interferon gamma (IFN-\(\gamma\)) and tumor necrosis factor alpha (TNF-\(\alpha\)), and antibody production.\textsuperscript{9}

Figure 2. Illustration of the components that play a role in the mechanism of immunologic tissue damage set in motion by dietary antigens. The antigens gain access to the submucosa by the paracellular route due to intestinal permeability a result from relaxed tight junctions, predominantly caused by zonulin, or to a lesser extent, lipopolysaccharide (LPS) in the event of bacterial exposure. The food antigen is first recognized and phagocytosed by antigen presenting cells (dendritic cells, B-lymphocytes or macrophages), whereby the antigen is displayed on surface of the cell via MHC II and presented to CD4\textsuperscript{+} helper T cells, orchestrator of the inflammatory response. Also shown is diamine oxidase (DAO), a protein produced by enterocytes of the intestinal mucosa,
whose function is to degrade histamine. In the absence or decrease production of DAO, the intestinal mucosa is prone to insults from histaminergic foods, resulting in the impairment of the microvilli.

Chronic exposure of immune cells to offending foods can result in overstimulation of immune cells that leads to antibody production, inflammatory reactions, and subsequently failure of self tolerance, whereby autoimmune disease ensues. Additionally intestinal and extraintestinal symptoms, that are non-specific to a definitive disease state, can also occur. An overwhelming amount of individuals battling with these symptoms either remain undiagnosed or they are incorrectly treated. Symptoms may be debilitating to the patient both physically and financially and result in a loss of productivity. The following table enumerates the financial impact of several symptoms on the United States of America health care system.

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Annual Total (Direct and Indirect) Costs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Migraine</td>
<td>$12 billion</td>
</tr>
<tr>
<td>Atopic Eczema</td>
<td>$364 million</td>
</tr>
<tr>
<td>Depression</td>
<td>$44 billion</td>
</tr>
<tr>
<td>Irritable Bowel Syndrome</td>
<td>$30 billion</td>
</tr>
<tr>
<td>Insomnia</td>
<td>$30-$35 billion</td>
</tr>
<tr>
<td>Pain</td>
<td>$560-$635 billion</td>
</tr>
</tbody>
</table>

Table 2. Various Symptoms and their impact on US health care costs.\(^ {48-53}\)

The complement pathway is a major effector mechanism of the innate immune system, with its primary function to destroy infectious agents, stimulate inflammatory response, and remove cellular debris.\(^ {54}\) It works collaboratively with the adaptive immune system. IgG is an effector molecule of the adaptive immunity, and its pleiotropic effect is demonstrated in its ability to modulate inflammation through the activation of the complement system, opsonization, and mediation of antibody dependent cell-mediated cytotoxicity (ADCC). There are four IgG subclasses, all of which are measured within the IgG/C3d assay. IgG4 predominantly correlates with delayed food allergy. IgG1 responds to new food antigens, IgG2 and IgG3 reacts to cell surface oligosaccharides of viruses, protozoa, and foods, which are potent allergic reactors.\(^ {55}\) Upon prolonged exposure to antigen, there is a class switch from IgG1 to IgG4. IgG1 is able to activate the complement pathway through the flexible CH2 domain, while, IgG4 does not activate complement.\(^ {56}\)
Figure 3. Depicts the three pathways of the complement system. The dense black arrows accentuate the classical pathway, which is utilized by the C3d/IgG study. Though all three pathways are able to generate the C3d fragment, the final cleavage product of C3, for simplicity the figure only shows its production by the classical and lectin pathway. C3d interacts with the CR2, which is expressed on neutrophils, follicular dendritic cells macrophages and B cells. When C3d binds CR2 it plays an important function in the cycle control of B cells by lowering the threshold for B-cell activation, thereby contributing to proliferation of antibodies.
Materials and Methods

The retrospective study was conducted on patients with symptoms, such as poor memory, poor concentration, anxiety, bloating, stomach pain, fatigue, joint pain, muscle aches and insomnia, to name a few. The population spanned an age range from 7 - 71 years, consisting of 9 males and 21 females. To determine the efficacy of the food sensitivity C3d/IgG test for its ability to manage patients with various symptoms, each patient’s medical record were analyzed, dating from 2009 to 2013. Patients also underwent detoxification protocols, and used various supplements. The subjects’ chief complaint was documented in the history of present illness from his or her first and subsequent office visits. Progression of symptoms were obtained from asking the patient to quantify how much their chief complaint had improved over six months to a year. A follow-up C3d/IgG test was performed during this interval. The second test was done after six months, because IgG exhibits a half-life of approximately 21 days with residual effector functions of about 2-3 months. Furthermore, IgG combined with antigen, forms an immune complex, and may remain in circulation for an extended period of time, which ultimately depends upon the magnitude of the antigen load and efficiency of the complement system to clear immune complexes. As in the case of cow’s milk allergy, IgG anti-milk can last for long as up to 9-12 months. A total of 132 foods were tested, including eight of the most highly allergic foods: milk, eggs, soy, fish, crustaceans (crab, lobster, shrimp etc.), wheat, peanuts, and tree nuts. The method used for analysis of patients’ blood samples was the indirect Enzyme Linked Immunosorbent Assay (ELISA). Samples were collected in serum separation tubes from the antecubital vein of patient’s preferred arm by a competent phlebotomist. The blood sample was allowed to sit for 10 minutes at room temperature, then subjected to a relative centrifugal force of 3200g for 20 minutes, and the serum fraction was collected. Serum samples were stored at -20 °C for optimal stability. The first day of sample preparation, all reagents, as well as, microwell plates were brought to room temperature before use. The ELISA plates were coated with a microarray of 132 different food antigens. The foods used were organic, and processed, as well as, standardized to enable coating onto ELISA plates. The plates and all the reagents used for the assay were produced by Brendan Bioscience LLC (3A Business Way, Hopedale, MA 01747) and have the patent # 8,309,318. The serum sample was diluted 1:10 in 1X solution of proprietary mixture and gently mixed on a rotating rocker platform to ensure even distribution. Afterwards, the sample was added to each well (50 µl/well) of the 96-microplate, with the exception of the last four wells, 50 µl of 1X proprietary mixture was added in column 11 and 12 reserved for the standard. The microplate was wrapped in parafilm and allowed to incubate 18-20 hours. The standard curve used was obtained from the semi-quantitative measure of HuIgG (human IgG): 0 ng, 25 ng, 7.5 ng and 1.25 ng to standardize inter and intra-assay readings.

During incubation, antibodies from the sample bind to food antigens on the ELISA plates. On the second day, the plates were washed three times with 1X proprietary buffer solution (Brendan Bioscience LLC) to remove unbound antibody followed by addition of the conjugate in each well (50 µl/well) including the last four wells in column 11 and 12 designated for the standard, then allowed to incubate for 65 minutes. The conjugate, a secondary antibody (also known as the labeled antibody), contains monoclonal anti-human-IgG-HRP (horseradish peroxidase) and monoclonal anti-human-C3d-HRP. The illustration below highlights the distinctive feature of the Ig/C3d test compared to a
The conjugate binds to only one domain common to all subclasses of HuIgG (1-4) and to HuIgG-C3d containing immune complexes, therefore contributing to the specificity (less cross-reactivity) of the test. Upon completing the 65 minutes incubation with the secondary antibody, the plates were washed two times to remove unbound conjugate, then OPD (ortho-phenylenediamine) substrate and an enzyme catalyst, a proprietary amalgam, detection solution with chromogenic properties, was added to each well (200 µl/well), including those designated as standards. This step of the assay was both light and time sensitive requiring only 15-20 minutes of incubation. Last, 3N sulfuric acid was added to each well (100 µl/well) to stop the reaction. Adding the sulfuric acid brought each well, including those designated as standards to a total volume of 300 µl. Plates were read at 492 nm using the Epoch microplate spectrophotometer (Biotek) to visualize the bound HuIgG and HuIgG-C3d immune complexes. After analysis of the data confidence limits were set that ensure a probability of ≥ 95% that a sample was either positive or negative for a particular food antigen. The assay was conducted per manufacturers instructions.

Figure 4. Illustrates the difference between conventional IgG and IgG/C3d test

Results

The patient’s antibody production to the offending foods decreased, and their symptoms either subsided or resolved. The offending foods were identified from the elevated C3d/IgG levels in patients’ sera against the various food antigens. Among the 30 subjects included in the study, there were 9 males and 21 females, with an age range from
7 – 71 years. 93% of the people tested demonstrated sensitivity to one of the eight foods that account for more than 90% of all allergic reactions documented worldwide. The eight foods are cow’s milk, egg, fish (all species of finfish), crustaceans (lobster, shrimp, crab), peanuts, tree nuts (almonds, cashew, walnuts etc.), soybean and wheat.

Initial and follow-up C3d/IgG testing are compared. The average time between the tests was 10.7 months. Between the initial and second test the patient was aware of high sensitivity foods to avoid and advised to make appropriate dietary changes. Figure 5 shows the scores of C3d/IgG initial tests across all patients (the number of foods scoring in the severe, high, and moderate ranges is reported). Figure 6 shows these corresponding scores for the second C3d/IgG test across all patients (an average of 10.7 months later). The reduction of detected C3d/IgG sensitivity is clearly illustrated between Figures 5 and 6.
Figure 5: Scores of C3d/IgG initial tests across all patients (the number of foods scoring in the Severe, High, and Moderate ranges is reported).
Figure 6: Scores of the second C3d/IgG test across all patients (an average of 10.7 months later).

In some cases, a third test was performed on patients (n=4). The results of the third test indicate that the C3d/IgG sensitivity result reduction is maintained past the second test as the dietary changes are continued, and is shown in Figure 7 and Figure 8 for the second and third tests, respectively.
Figure 7: The subset of participants’ second C3d/IgG test results in which a third test was also performed.
The reduction of detected C3d/IgG sensitivity between the initial and second test is again clearly illustrated in Figure 9, which summarizes the average and standard deviations across the two data sets. A t-test statistical analysis using the two-paired samples between the first and second tests was run to determine if the difference was significant. The p-values are 1.56E-06, 0.007, and 0.001 for the severe, high, and moderate test results between the initial and second pair. A p-value below 0.05 indicates a strong presumption that the difference in the results is significant. Hence, the difference between the initial and second test results can be strongly presumed significant.
Figure 9: Summary of the average, standard deviations, and p-values for C3d/IgG sensitivity scores across the initial and second tests for paired patient results. Clinical manifestations are reduced from the initial to the second test dates.
<table>
<thead>
<tr>
<th>Complaint</th>
<th>Number Of Patients Reporting For Initial Test</th>
<th>Number Of Patients Reporting For Second Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Memory/Concentration</td>
<td>22</td>
<td>3</td>
</tr>
<tr>
<td>Anxiety/Mood/Depression</td>
<td>20</td>
<td>3</td>
</tr>
<tr>
<td>Bloating/Stomach Pain</td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td>Fatigue</td>
<td>18</td>
<td>4</td>
</tr>
<tr>
<td>Joint Pain / Stiffness / Swelling</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td>Muscle Aches</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td>Craving Sugar</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>Sleeplessness/Insomnia</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Lightheaded/Dizzy</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>Allergies/Sinus</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>Cold Intolerance</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>Inability to lose weight</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>Libido/Impotence</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Constipation</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Halitosis</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Tearing Eyes</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Brittle Nails/Dry Skin/Dry Mouth</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Bruising</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Headaches</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Irregular Heart Beat</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Numbness Hands/Feet</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Sinus Problems</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Thyroid</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Eczema</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 3. Highlights the reduction in the number of symptoms reported by patients in the initial test compared to the second test, after eliminating severe, high, and moderate reactive foods.

The foods that demonstrated the greatest reactivity in the C3d/IgG test are shown in Figure 10, which shows the foods that ranked as Severe or High reactivity with the greatest frequency.
Discussion

The aim of the study is to assess the efficacy of C3d/IgG testing based on food elimination to decrease various symptoms. Patients who complied with the avoidance of anti-C3d/IgG dietary antigens demonstrated statistically significant reduction in C3d/IgG testing sensitivity, and marked reduction in symptoms that they reported before beginning the diet. Besides, the elimination of the offensive foods, which was the principal cause of the patients’ improvement, subjects were recommended detox and supplements to enhance the rebuilding of the gastrointestinal wall integrity. This corroborates other studies, showing that elimination of the offending foods identified to be inflammatory using the C3d/IgG tests is effective in mitigating and treating chronic symptoms that are characteristic of various disease states. A high level of significance found in this intervention is likely from a combined method of testing C3d and all four types of IgG simultaneously. With complement accounting for the preponderance of the inflammation, causing the patient’s symptoms, measuring C3d, a cleavage fragment of C3 shows promise as a useful inflammatory biomarker in clinical applications. For example, patients with gastrointestinal symptoms, involving pain, diarrhea, constipation and bloating typical of irritable bowel syndrome, and frequent headaches typical of migraine, observed noticeable improvement when placed on an individualized diet with elimination of the offending foods. This study supports the validity of the C3d/IgG tests to identify the severity of food allergies and as an effective tool in clinical intervention.

Confounding variables include: 1) patients taking pharmaceutical agents to alleviate symptoms; 2) socioeconomic challenges preventing patients from obtaining and sustaining themselves on the recommended dietary regimen; 3) level of the patient’s knowledge and interest about their symptom/disease and therapy; 4) Symptom data was
collected by patient response, which is subjective. Further prospective studies are warranted, with a larger subject population, and a revised methodical approach to control for confounding factors and biases.

With numerous studies documenting the pathogenesis of various diseases associated with intestinal permeability, more attention should be placed on the contributive factors of this mechanism. Microbial pathogen, alcohol, stress, inflammatory mediators are contributive factors of intestinal permeability alongside antigenic foods. According to the United States Department of Agriculture (USDA), a study conducted over the course of 2006-2008, showed that the average American age 15 and older spent approximately 2.5 hours eating or drinking each day. In another article released by the USDA’s Economic Research Service, the average American consumed approximately 2000 pounds of food in 2011. These data further provides a reference how food could possibly contribute to many symptoms and conditions, due to the sheer magnitude consumed, constitutively keeping the immune system activated, perpetuating chronic inflammation. With all the stratagem and treatment plans put forth in various formulations to treat symptoms of bloating, abdominal pain, diarrhea, constipation, fatigue, migraines, headaches cognitive dysfunction, depression, myalgia, joint pain, sinusitis, eczema and urticaria this study have demonstrated strong, supportive data to warrant the use of C3d/IgG tests in clinical intervention in managing patients of diverse symptoms and to lower total inflammatory load.

Conclusion

Patients who complied with avoiding C3d/IgG anti-food antigens experienced significant improvements in their chief complaints. The C3d/IgG testing may be an important clinical tool in the management of symptoms related to food sensitivities, such as bloating, abdominal pain, diarrhea, constipation, fatigue, migraines, headaches cognitive dysfunction, depression, myalgia, joint pain, sinusitis, eczema and urticarial. Overall, patients’ food sensitivity was inversely proportional to their symptoms. Patients felt better when C3d/IgG food sensitivity was low, demonstrating food removal based on the C3d/IgG test is an effective approach in patient care.
References


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