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# CELIAC DISEASE and the Molecular Detection of Imbalances Through the use of a High-Precision Method

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**Abstract**

Celiac disease (gluten enteropathy, enteropathy, and J-Herter-Heubner disease) - pathological disorders of the intestine, in which there is an intolerance to gluten. As 70 samples were collected from patients, the aim of which was to know the molecular detection of the disturbances occurring through the use of the accurate method, which is SYBR Green technicality with melting curve analysis on genomic DNA Isolated and for the control samples, it consisted of 30 samples, The increase in mortality from all diseases in the group compared to the initial period from 62 to 77% was a limited value without a clear correlation and only confirmed the trend of increase in total deaths from all causes from 1.37 to 1.57, excluding deaths from cardiovascular disease. , Although in half of the cases it was related to celiac disease. In the structure of mortality, an increase in respiratory diseases is observed during the period of application of serological diagnostics

**Introduction**

Celiac disease is a multifactorial disease that develops when parietal digestion and malabsorption in the small intestine are impaired, caused by certain foods containing gluten protein (gluten) and related cereal proteins. Has a mixed autoimmune, allergic, hereditary genesis. Fortunately, the disease in severe form is rarely diagnosed, but the gene responsible for the predisposition to celiac disease is noted quite often, which contributes to the

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manifestation of the disease in the population in about 0.5-1% of the population of varying severity. Severe celiac disease tends to be associated with high mortality, while milder forms of celiac disease are less disastrous with a gluten-free diet.[1]The development of modern serological diagnostic methods over the past decade has made it possible to more accurately and at early stages identify the disease. The introduction of reliable serological tests for celiac disease into everyday practice has contributed to the development of a strategy for its early diagnosis and stratification of disease risk. At the same time, at the moment there is no data on how this affected the overall survival of patients , whether there is a difference in survival among patients with moderate celiac disease. The hypothesis that milder cases detected early in the course may be Which is related to death rates, required proof.[2]

**Non-celiac sensitivity to gluten**

Patients encountering help of both gastrointestinal and different indications when changing to a sans gluten diet (BGD), barring celiac sickness and interceded wheat hypersensitivity (PA), are considered to have Non-celiac gluten affectability" (HCG) [3]. Regardless of the successive abstract improvement of indications which patients experience at change, the aftereffects of clinical preliminaries (gluten versus fake treatment) in the present the second is conflicting [4], which doesn't permit us to pass judgment on reality with sureness the recurrence of this disorder. Right now, the best way to recognize a particular food affectability (when a particular mechanical test isn't free) is playing out a twofold visually impaired fake treatment controlled cross-diet incitements utilizing wheat protein containing at least others substances, (for example, fructosans) that can cause manifestations. Not in the least less, at such incitements there are nocebo impacts, an ordinary marvel, which should be considered when deciphering the outcomes .[5,6] Moreover, on the grounds that there are still no particular ones proof that gluten is answerable for the beginning of manifestations and is obscure HLA qualities assume a significant part in deciding gluten defenselessness Gluten prejudice connected to HLA haplotypes, hazard of celiac sickness is brought about by two hereditary loci HLA-DQ2 and HLA-DQ8, which take support in the last insusceptible reaction. Appropriately, if the investigation shows that the analyzed individual doesn't have these qualities, which implies that he can't have celiac sickness and no compelling reason to expose him to promote examination [8,9].The investigation is a helpful method to analyze, in light of the fact that doesn't need, in Unlike histology, it is hard to acquire a biomaterial. This technique is particularly valuable when if there is motivation to presume gluten bigotry, however blood tests for antibodies are negative, and biopsy is bothersome (because of burden or narrow mindedness strategies) .[10] Indeed, even limited quantities of gluten can trigger

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indications. In the event that you don't eliminate gluten from your eating routine, the villi in the covering of the small digestive system will start to grow or even vanish, and subsequently won't ingest the supplements in the sum they need, which will eventually prompt stomach related uneasiness [11] .Recognize the work of art, indicative and subclinical types of celiac illness. These structures are related with harm to the mucous layer of the small digestive system. Also, there is a supposed conceivable celiac illness, which is typically found by chance during an ordinary biopsy of the intestinal mucosa [12].

**Differential diagnosis**

Differential diagnosis of celiac disease, wheat allergy and non-celiac hypersensitivity to glutenOther causes of enteropathy (atrophy of intestinal villi): chronic giardiasis, tropical sprue, protein deficiency, mental anorexia, food hypersensitivity (changes are usually focal), viral (including HIV) and bacterial (e.g. tuberculosis) infections, dysbacteriosis, Whipple's disease, postponed radiation therapy, immune deficiency (, Crohn's disease, ulcerative colitis, small bowel lymphoma [13].

**Dysfunctions**

It is a genetically determined dysfunction in the small intestine linked to a deficiency of enzymes that break down gluten peptide. Gluten (glutenopectin) is the alcohol-soluble protein residue from grains after starch and other sugars have been extracted from them. It is characterized by high resistance to digestive proteolytic enzymes in the digestive system. As a result, peptides that are toxic to intestinal cells are formed and lead to their damage[14].There are a number of additional factors required for the development of celiac disease. In particular, an intestinal infection can be a trigger for the development of celiac disease in an predisposed person. Another factor is the elevated activity of a number of connective tissue enzymes, primarily tissue transglutaminase, in the intestinal wall [15] . Secondary lactase deficiency, which develops against the background of celiac disease, is a factor that leads to acidification of the contents of the small intestine, and contributes to the development of the disease[16].A predisposition to specific diseases is determined genetically, which is often associated with the HLA system. For a portion of the population - carriers of some human leukocyte antigen characteristics - there is a risk of developing one or another disease. A similar genetic program is achieved by HLA antigens present on the cell membrane and being a manifestation of the activity of the corresponding genes [17].Class II HLA genes are expressed in B lymphocytes, activated T lymphocytes, monocytes, macrophages, and dendritic cells. Protein products encoded by HLA class II genes possessing

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strong antigenic properties play an important role in regulating the recognition of foreign agents and are essential participants in many immune reactions, and play a key role in the development of the acquired immune response. Another aspect of a person's resistance to infection could be autoimmune diseases [18].Within the HLA class II genes, sites of prime importance in clinical practice are distinguished: DQA1 (25 allelic variants), DQB1 (57 allelic variants).Patients with celiac disease show specific immune responses from birth. Only a small percentage of carriers of human leukocyte antigen traits (according to various estimates, about 3% of the total) actually suffer from celiac disease. That is, determining the genetic predisposition cannot confirm celiac disease and is not even considered an indication for serological examination[19].At the same time, the absence of distinct allelic combinations in the HLA genes makes it possible to exclude the diagnosis of celiac disease, primarily in the risk group for the development of this disease.

Molecular genetic markers of a class II HLA system that determine susceptibility to celiac disease in a European population

Alleles of HLA class II genes Heterodimer (protein)

Place DQB1 instead of DQA1

02:01 (02:01 *) 05:01 DQ2(%95)

03:02 03 DQ8(%5)

Thus, the presence in the genome of the DQB1 * 02: 1 haplotype; DQA1 * 05:01 (* 02:01) or DQB1 * 03:02; DQA1 * 03, which leads to the synthesis of DQ2 or DQ8 heterodimers, is required but insufficient for the development of celiac disease in humans.

The designation of a gluten-free diet, that is, a nutritious diet that excludes foods containing cereal prolamine, removes the triggers for the development of autoimmune inflammation. When a gluten-free diet is prescribed, the morphological changes characteristic of celiac disease disappear, the titers of antibodies to tissue transglutaminase, gliadin and their fragments are lower than those detected within 1-6 months and become negative in all patients after one year of adherence to the diet [20].

**Methods:**

The methodology relied on the development of the pcr test, through the adoption and use of SYBR Green technology, and with the development taking place at the present time, we find that the fusion method in the analysis of the genomic acid curve obtained by 70 patients in addition to that was used HLA-DQA1 * 05 and HLA- DQB1 * 02 for examination As for the purpose of the research uses was to determine predisposition to celiac disease, and the study was scheduled in suspicious cases, when the diagnosis of celiac disease was determined

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(detection of HLA DQ2, DQ8 makes the diagnosis more likely, and their absence eliminates Doubt) [21].

Resistant celiac disease is characterized by a lack of response (clinical, serological and morphological) to the agliadin diet. Refractory form celiac disease (with the unconditional exclusion of violations of agliadin diet) is not at all so and requires further diagnostic research. Refractory materials celiac disease is diagnosed on the basis of persistence or reappearance of clinical symptoms of celiac disease with strict adherence to a gluten-free diet within 6-12 months, in the absence of other clinical causes of symptoms, including tumors. The incidence of refractory celiac disease in patients with celiac disease is little known, however, according to available data, it is about 1%.

Definition of HLA class II - DQ2, DQ8, DQAl * 05 with celiac disease by polymerase chain reaction. Genetic research includes determination The patient possesses characteristic HLA-DQ2 / DQ8 alleles with negative genetic typing results having a high predictive value, allowing celiac disease to be excluded. HLA-DQ2 / DQ8 genotyping should be used in order to exclude celiac disease, before deciding whether gluten loading is needed. The genetic typing can be used to exclude the diagnosis of celiac disease in difficult diagnostic situations the genetic markers in this case are determined by the fact that it does not depend on whether or not the patient follows a gluten-free diet at the time of the study[22].

**isolation of nucleic acids in routine laboratory practice use the three most common approaches:**

- Express methods (used for DNA extraction) are based on temperature lysis of cells and subsequent centrifugation, as a result of which insoluble components settle at the bottom of the tube, and the supernatant (supernatant) containing DNA is used for PCR.
- The method is easy to use, takes no more than 10-15 minutes, provides maximum DNA yield, but is ineffective when working with material containing , high amount of impurities and PCR inhibitors. Also, the method cannot be used for RNA isolation, since RNases are resistant to heat.
- If need to use express methods with "complex" samples it is possible to introduce additional methods of purification and concentration of the material, for example, in the case of excess mucus in the sample (for sputum and ejaculate, bronchoalveolar

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lavage, bronchial lavage water, tracheal lavage, synovial and pleural fluid), preliminary washing and treatment with mucolytics are advisable More efficient method for the isolation and purification of nucleic acids (NK) when working with such material, there may be the use of sorbents or strong chaotropic agents.

- Sorbent methods of isolation - differential sorption of nucleic acids (isolation of both DNA and RNA is permissible) on a solid carrier (more often

total: silica gel with increased negative charge or modified surface, glass beads, diatomaceous earth, Inhibitors and others components of the clinical material remain in solution. In addition to chaotropic salts in the lysis buffer, detergents are most often present that contribute to dissolution and lysis of proteins. Silica with NC is precipitated by centrifugation, and the supernatant with PCR inhibitors is removed. A series of subsequent washings provides a highly purified NK preparation. The currently relevant modification of the sorbent method – isolation on magnetic particles in manual or automatic modes , The use of magnetic solid carriers has a number of advantages over non-magnetic separation methods[23].

Table 2 - PCR cycles that used for Tm setup

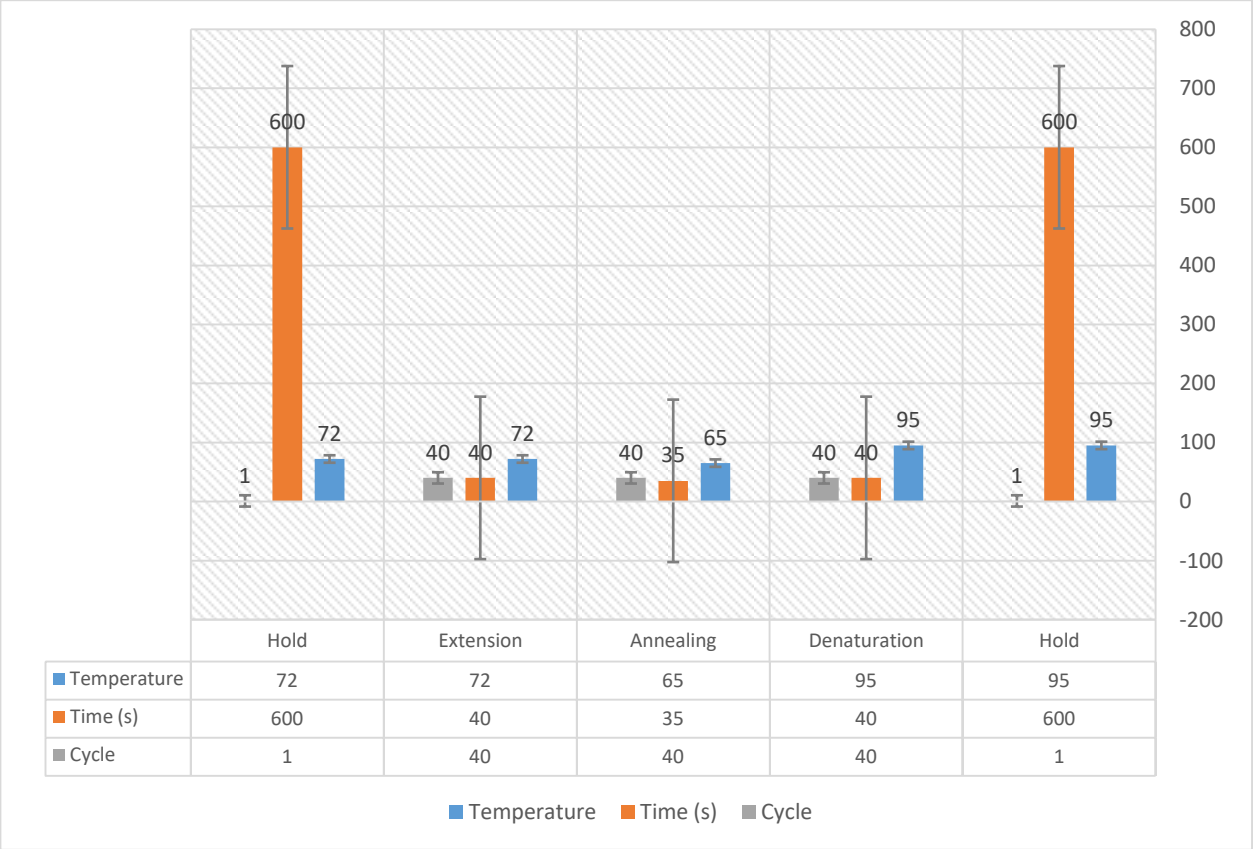

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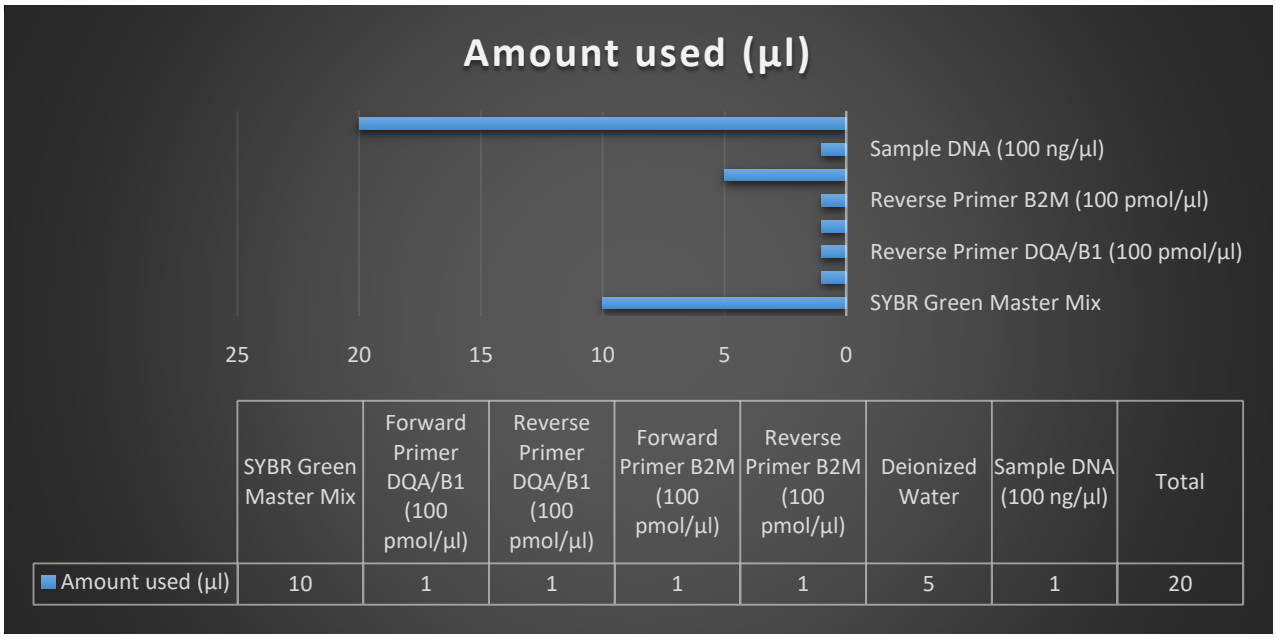
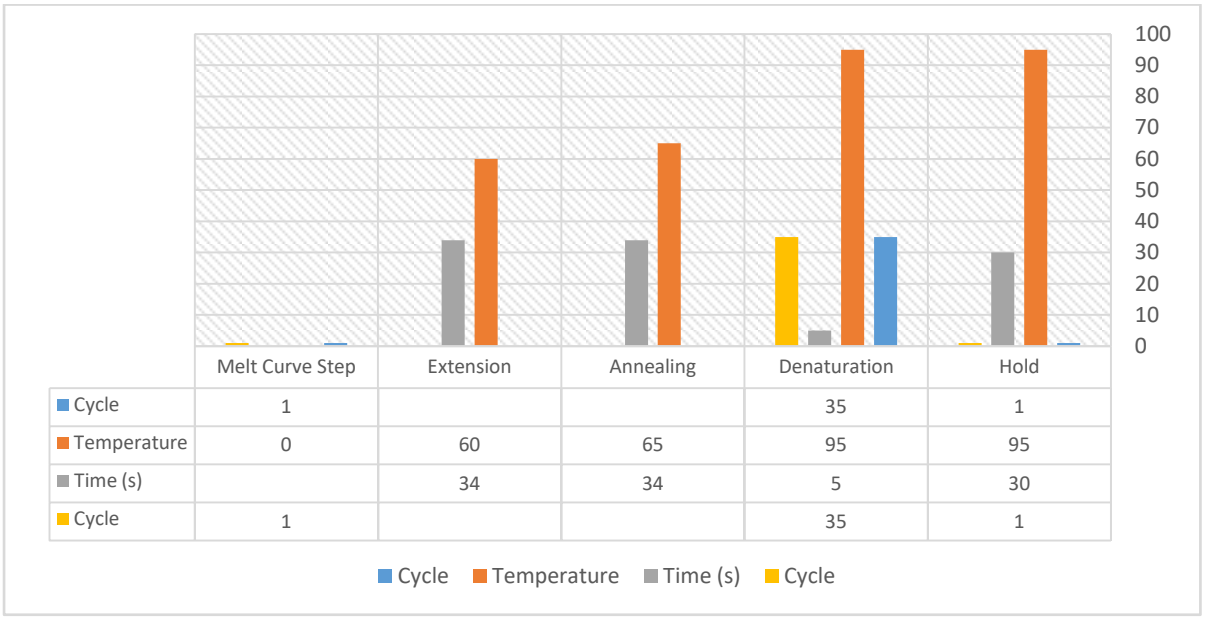
**Table 3.** The amount of mixed per sample




| | SYBR Green Master Mix | Forward Primer DQA/B1 (100 pmol/µl) | Reverse Primer DQA/B1 (100 pmol/µl) | Forward Primer B2M (100 pmol/µl) | Reverse Primer B2M (100 pmol/µl) | Deionized Water | Sample DNA (100 ng/µl) | Total |
|---|---|---|---|---|---|---|---|---|
| Amount used (µl) | 10 | 1 | 1 | 1 | 1 | 5 | 1 | 20 |

Table 4. Real-time PCR cycle conditions




| | Melt Curve Step | Extension | Annealing | Denaturation | Hold |
|---|---|---|---|---|---|
| Cycle | 1 | | | 35 | 1 |
| Temperature | 0 | 60 | 65 | 95 | 95 |
| Time (s) | | 34 | 34 | 5 | 30 |
| Cycle | 1 | | | 35 | 1 |

**results: - result of patient**

| | | |
|---|---|---|
| GI symptoms | 52 (69.4%) | Heartburn |
| | 36 (48%) | Diarrhea |
| | 30 (40%) | Nausea and Vomiting |
| | 62 (82.7%) | Bloating |

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| | | |
|---|---|---|
| Non-GI symptoms | 51 (68%) | Weight Loss |
| | 43 (57.4%) | Anemia |
| | 31 (41.4%) | Bone problems |
| | 46 (61.4%) | Neurological problems |
| | 4 (5.4%) | Infertility |
| | 37 (49.4%) | Aphthous |
| | 18 (24%) | Skin problems |
| Histology | 7 (9.3%) | Marsh I |
| | 9 (12%) | Marsh II |
| | 18 (24%) | Marsh IIIa |
| | 13 (17.3%) | Marsh IIIb |
| | 28 (37.4%) | Marsh IIIc |

**Discussion**

The HLA typing for celiac disease has been developed using various methodologies. These diagnostic methods have their own advantages and disadvantages For example, two decades ago, Otten and her colleagues used the serological method for HLA-DQ typing. * This method has varying difficulties and also low accuracy With the growth of molecular approaches, Michalski et al. Used the method restriction fragment length polymorphism (RFLP-PCR) for haplotype determination HLA-DQ in patients with Celicia. 89 Although this method has a high accuracy in comparison with the serological method, it was long, laborious and needed gene sequencing for verification. After that, developed the Single Specific Primer-PCR (SSP-PCR) technique, which made it possible to identify all alleles are HLA-DQ. SSP-PCR is a sensitive approach, but

application required a post-PCR process. Megiorny et al. Later identified the HLA-DQ alleles in patients using primers developed As these primers overlap with other haplotypes such as DQ3, DQ7 and DQ9, then there was a risk of false positives , A rapid and sensitive method to detect specific human lymphocyte antigen (HLA) class II alleles associated with celiac disease , Another molecular technique that has recently been used for typing HLA, includes Sequence-Based Typing (SBT), sequence-specific hybridization of oligonucleotide probes (SSOPH), Reference Strand mediated Conformation Analysis (RSCA) and PCR-SSP. These methods provide powerful tools for identifying HLA alleles, but they have limitations such as high cost (RSCA), labor intensity (SSOPH) and unsuitable for large scale (SBT) population. Many of these methods have been optimized and entered the market as a commercial kit such

as the Olerup SSP-Kits, however, its cost today is high .contain fewer processing steps and are very sensitive, allows to identify the disease before the manifestation of clinical symptoms and to identify predisposition to the development of celiac disease.

Worldwide there is a large variety of PCR kits designed for To identify celiac disease. According to Rouvroye compared to 3 Various groups (XeliGen group, MLPA group, CeliaSCAN group), have come to the conclusion that all groups correctly identify genes for celiac disease risk. Laboratory resources and the intended use should determine the preference for any of the HLA-DQ identification mapping combinations.for the first time, the identification of HLA class II alleles - DQ2, DQ8, QAl * 05 in celiac disease by PCR was introduced. The search for clinical efficacy has shown that this method is effective in determining the alleles of celiac disease.

**Conclusion**

- Determination of DQ2, DQ8, DQAl * 05 HLA class II by PCR has a high sensitivity and specificity for identifying the corresponding alleles genes of the histocompatibility system.
- The diagnostic value of genotyping lies in the possibility application of the method in complex diagnostic cases and allows you to exclude a diagnosis of celiac disease, with conflicting serological test results, and biopsy, respectively, the method does not provide for its use as primary test to verify the diagnosis.
- for suspected celiac disease depend on the frequency of cases of questionable first-line diagnostic test results (0.75% to 9.26%) of cases

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