The Role of celiac disease antibodies in the follow up of Patient on Gluten free diet

El papel de los anticuerpos de la enfermedad celíaca en el seguimiento del paciente con una dieta sin gluten

[1]Abbas Mohammed Hussein Alshebani, [2]Zainab Salih Abdalhamza, M.B.Ch.B.
[1]Department of pediatric, college of medicine, University of AL-Qadisiyah
[2]AL-Diwaniaih maternity and children hospital

Abstract

Background: The detection of autoantibodies directed against tissue transglutaminase have a well-established role in the diagnosis of celiac disease and in long term follow up.

Aim of study:

1. To determine the role of celiac disease antibodies, in follow up the response of the patient with CD while he is on GFD.
2. To study the difference in the response between patients with GIT and non-GIT manifestations.

Patient and method: across sectional study was conducted from 1st of Feb. 2015 to 1st of May 2016,123 patients were involved in this study, and includes all patients that consult the outpatient clinic in maternity and Children Hospital in AL-Diwaniah Governorate, complaining of chronic diarrhea or failure to thrive, patients with pica or anemia without clear cause, patient with short stature without obvious pathology, and celiac patients who diagnosed previously, their age between 9 month to 12 years. From each patient, five ml of venous blood aspirated and sent to the lab where Enzyme Linking Immunosorbant assay (ELISA), measurement of auto antibodies specific for celiac disease (anti-endomysial IgA, IgG and anti-tissue transglutaminase IgA, IgG) was done.

Result: there was significant decrease in all enzymes level after several months of gluten free diet.

Conclusion: Celiac disease can present with variable manifestations, the follow up of celiac pt. may be important to detect the adherence of the pt.to gluten free diet.

Keywords: autoantibodies, dietetic changes, Celiac Disease, enzymes level

Resumen

Antecedentes: la detección de autoanticuerpos dirigidos contra la transglutaminasa tisular tiene un papel bien establecido en el diagnóstico de la enfermedad celíaca y en el seguimiento a largo plazo.

Objetivo de estudio:

1. Para determinar el papel de los anticuerpos de la enfermedad celíaca, en el seguimiento de la respuesta del paciente con EC mientras está en GFD.
2. Estudiar la diferencia en la respuesta entre pacientes con GIT y manifestaciones no GIT. Paciente y método: se realizó un estudio transversal desde el 1 de febrero de 2015 hasta el 1 de mayo de 2016. Participaron en este estudio 1223 pacientes, e incluye a todos los pacientes que consultan a la clínica ambulatoria de maternidad y al Hospital de Niños de la Gobernación de AL-Diwaniah, quejándose. De diarrea crónica o falta de crecimiento, pacientes con pica o anemia sin causa clara, pacientes con baja estatura sin patología evidente y pacientes celíacos que diagnosticaron previamente su edad entre 9 meses y 12 años. De cada paciente, se aspiraron cinco ml de sangre venosa y se enviaron al laboratorio donde se realizó la prueba de inmunoabsorción enzimática de enlace enzimático (ELISA), la medición de los autoanticuerpos específicos para la enfermedad celíaca (IgA anti-endomisial, IgG y anti-tejido transglutaminasa IgA, IgG).

Resultado: hubo una disminución significativa en todos los niveles de enzimas después de varios meses de dieta sin gluten.

Conclusión: la enfermedad celíaca puede presentar manifestaciones variables, el seguimiento de la enfermedad celíaca. Puede ser importante detectar la adherencia de la dieta libre de gluten.

Palabras clave: autoanticuerpos, cambios dietéticos, enfermedad celíaca, nivel de enzimas.

Celiac disease (CD) is a health disorder that is related to certain genetic events which affect the immune system resulting in showing such condition. CD is also linked to the effects of gluten and prolamins on susceptible humans. The disease is well-understood for its clinical features according to gluten-feeding history plus detecting ACDAs, HLA-DQ2, DQ8 haplotypes, and enteropathy-based diagnosis. Those ACDAs include autoimmune Abs such as AnG and AntTG. Gluten and prolamins are present in high amounts in wheat and barley. According to some reports, oat was noticed to have some prolamin-related CD effects involving the T-cells in the mucosa, but in general oat is safe. The disease occurrence is about 1% of the total biopsy-examined conditions[1]. Individuals who have the major histocompatibility complex class II genes plus the allele that codes for the human leukocyte antigen (HLA) presented by HLA-DQ2 and HLA-DQ8[2]. Environmental effects apply some effects in developing CD; however, gluten plays the most important role in this process in which gluten stimulates innate immunity that eventually induces inflammatory-based destruction to the GIT mucosa. Removing gluten from diets adds successful improvement to the health status of CD patients[3,4]. This was proven by some studies that involved biopsy and tissue-culturing tools[5,6]. There are some methods to diagnose CD, but the main tool is the histopathological changes that affect the GIT layers such as mucosa. These changes may include atrophy that affects villi, hyperplasia that affects crypts, and excessive inflammation. The second most important way to detect this disease is using ACDAs that work against certain antigenic compounds such as AnG and AntTG. However, in certain cases serological tests (STs) may become the first choice before doing biopsies using GIT endoscopy. In most cases, the STs may face some challenges as they have low sensitivity and specificity capacities leading to false positive results. In some patients who have different GIT health issues may have some serum titers of CD antibodies which may misdiagnose as celiac disease[7-9]. Some developed STs relying on gluten-dependent IgA-class R1-type reticulin (ARA) and endomysial autoantibody (EMA) showed strong results that have accuracy rates of 90%[10-13]. For better management of CD, children have to be on GDD and to be visited under scheduled time points to monitor their health status exposure to a gluten rich diet (GRD) after the 2-year exposure to the GDD on the health status of those patients.

Aim of study: To determine if serial measurements of celiac disease autoantibodies confirm adherence to gluten free diet.

Patients and methods. This cross sectional study was conducted from 1st of Feb. 2015 to 1st of May 2016 and involved all patients that consult the outpatient clinic in Maternity and Children Hospital in AL-Diwaniah governorate complaining of chronic diarrhea or failure to thrive and patients with anemia or pica that were not responding to treatment and patients with short stature without obvious cause in spite of full assessment also any atypical symptoms that suggest celiac disease and their ages between 9 month to 12 years. Thorough history and physical examination were performed to all patients with measurement of weight, height and head circumference and plotted on chart that suitable for sex and age, all patients have GSE and CBC, Blood film and serum iron, IBC, thyroid function test, growth hormone.

123 patients were involved in this study 25 of them were excluded (11 of them diagnosed as giardiasis, 7 diagnosed as secondary lactase deficiency, 1 patient diagnosed as inflammatory bowel disease, 6 families refused to do more investigations) 98 patients were assessed for possibility of celiac disease (sent for anti –tissue trans glutaminase and anti endomysial antibodies and HLA assessment, which was performed by a student of master´s degree at the College of Medicine University of Al Qadisyah, who helped us in doing HLA system. According to the following ranges of anticeliac antibodies:

Normal range < 12 U\ml

Equivocal range 12-18 U\ml

Positive result > 18 U\ml

From each patient five ml of venous blood aspirated and sent to the lab in aldiwaniah hospital where Enzyme Linking Immunosorbent assay (ELISA) assay were used for detection and measurement of auto-antibodies specific for (anti-endomysial IgA, IgG and anti-tTG IgA,IgG) (Aesku Diagnostics Microform ring 2. 55234 Wendelsheim Germany) were used in this work.

A buffered solution of the antigen to be tested for is added to each well of a microtiter plate, where it is given time to adhere to the plastic through charge interactions.

- A solution of nonreacting protein, such as bovine serum albumin or casein, is added to well (usually 96-well plates) in order to cover any plastic surface in the well which remains uncoated by the antigen.
- The primary antibody with an attached (conjugated) enzyme is added, which binds specifically to the test antigen coating the well.
- A substrate for this enzyme is then added. Often, this substrate changes color upon reaction with the enzyme.
- The higher the concentration of the primary antibody present in the serum, the stronger the color change. Often, a spectrometer is used to give quantitative values for color strength. 63 patients were positive for both tests: more than 18 unit/ml and HLA DQ2, DQ8. Full instructions about the diet of the patient was dis-

cussed with their family and mobile phone No. obtained from all patients caregiver and to be checked every three months with full history, examination, measurements and all patients data were recorded.

After 3 months, 18 patients were excluded (8 lost follow up and 10 did not follow strictly the gluten free diet) so 45 patients were included and the results were obtained.

Statistical analysis.

Discrete variables presented as number and percentage, continuous variables presented as mean ±SD (standard deviation). Chi square and Fischer´s Exact probability test were carried out to examine the association between signs and symptoms and level of enzymes. Student t test was applied to test the difference between normal and abnormal level of enzymes during periods of follow up. P-value <0.05 was consider significant. The program was used are SPSS version 18.

The results: About 95% of cases were presented and diagnosed under the age of three years.

Table 1. Classifications of patients according to the age of diagnosis:

Age in months	No. of pt.	%
9-18	33	73.3
19-36	10	22.2
37-60	2	4.4
>60	0	0
Total	45	100

Most patients were males figure 3: classifications of pt. according to the residence: rural (30), urban (15)

Figure 1: classifications of patients according to the sex: male (28) female (17)

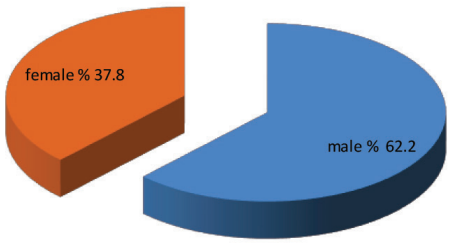
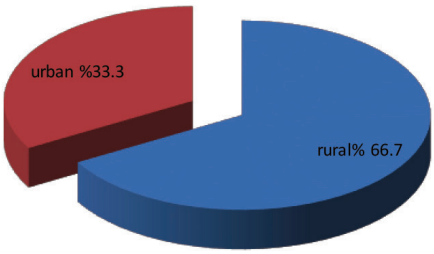


Figure 2: classifications of pt. according to the residence: rural (30), urban (15)



People in rural areas are more affected than those in urban areas

Table 2: classifications of pt. according to the clinical presentations: Most patients presented GIT manifestations.

Table 2: classifications of pt. according to the clinical presentations:

Clinical features	No.	%
GIT	38	84.5
Non GIT		
SS	2	4.4
Pica	5	11.1

Table 3 presents the enzyme level after three months of gluten free diet:

Table 3: enzyme level after three months of gluten free diet:

No.	Normal enzyme level	Abnormal enzyme level after 3 month
GIT symptoms	24	14
Non GIT		
SS	0	2
Pica	2	3

Table 4. P-value of enzymes levels after three months of gluten free diet there were significant decrease in all enzymes levels after three months of gluten free diet (p-value <0.05)

Table 4. P-value of enzymes levels after three months of gluten free diet

	After 3 month	N	Mean	Std. Deviation	Std. Error Mean	p-value
IgG tTG	normal	26	9.5000	1.42127	.27873	0.001
	abnormal	19	66.4737	39.93379	9.16144	
IgA tTG	normal	26	9.6154	1.16883	.22923	0.0042
	abnormal	19	57.0000	41.50904	9.52283	
IgG EMA	normal	26	9.3077	1.12318	.22027	0.0012
	abnormal	19	82.6842	66.00089	15.14164	
IgA EMA	normal	26	8.7692	1.06987	.20982	0.0031
	abnormal	19	73.9474	56.86385	13.04546	

After three months of gluten free diet most patients presented with GIT manifestations have normal enzymes while in patients whose presented with non GIT symptoms especially those with short stature and pica still have high enzymes levels.

Table 5: enzyme levels after six months of gluten free diet:

Table 5: enzyme levels after six months of gluten free diet:		
No.	Normal level	Abnormal level
GIT(13)	9	5
Non GIT(6)		
SS	1	1
Pica (3)	2	1

Table 6: p_value for enzyme levels after six months of gluten free diet: there were significant decrease in all enzymes levels after six months of gluten free diet (p-value <0.05) after six months of gluten free diet only four patients from those with GIT symptoms (35patients) still show abnormal enzymes levels while three from ten patients with non-GIT symptoms have high enzymes level.

Table 6: p_value for enzyme levels after six months of gluten free diet:						
	After 6 month	N	Mean	Std. Deviation	Std. Error Mean	p-value
IgA EMA	normal	12	9.1111	.78174	.26058	0.001
	abnormal	7	93.1429	48.08128	18.17302	
IgA tTG	normal	12	9.2222	.66667	.22222	0.002
	abnormal	7	62.7143	54.34677	20.54115	
IgG EMA	normal	12	9.2222	.83333	.27778	0.002
	abnormal	7	98.2857	48.58571	18.36367	
IgA tTG	normal	12	9.0000	.86603	.28868	0.003
	abnormal	7	71.0000	45.99638	17.38500	

Table 7: enzymes level after nine months:

Table 7: enzymes level after nine months:		
No.	Normal enzyme	Abnormal enzyme
GIT (4)	3	1
Non GIT		
SS(1)	0	1
Pica (1)	0	1

Table 8: p_value for enzyme levels after nine months of gluten free diet: there was significant decrease in all enzymes levels after nine months of gluten free diet (p-value <0.05)

After nine months of treatment nearly all patients with GIT and non-GIT symptoms, abnormal levels were still found in three patients: one with GIT and two with non-GIT symptoms. The patient with short stature showed good linear increment (about 8cm/year) and had a history of very good strict gluten-free diet, about the patient with pica, the symptom disappeared after three months of treatment but the enzyme levels remained elevated even after one year.

Table 8: p_value for enzyme levels after nine months of gluten free diet:						
		N	Mean	Std. Deviation	Std. Error Mean	p-value
IgG EMA	Normal	4	9.7500	.50000	.25000	0.001
	abnormal	3	88.0000	11.00000	6.35085	
IgA EMA	normal	4	9.2500	.95743	.47871	0.02
	abnormal	3	87.6667	11.50362	6.64162	
IgG tTG	normal	4	9.0000	.81650	.40825	0.004
	abnormal	3	62.3333	16.80278	9.70109	
IgA tTG	normal	4	9.5000	1.29099	.64550	0.005
	abnormal	3	66.0000	29.10326	16.80278	

Our study shows that most patients may be diagnosed before the age of three years, in agreement with many studies done around the world as in Sweden by Anneli et al[14], who did screening for celiac in children less than 3years of age, and stated that the high gluten intake among very young Swedish children may increase the prevalence of CD. Also male is more affected by CD than female, but this disagrees with many studies which explained that CD seems to be more frequent in females than males as study was done at 2012 in Iran by Mehrdad et al.[15] and children who live in rural area are more involved by CD than those in urban area and this may be explained by the fact that children in rural areas have exposed to gluten earlier than in urban areas and also there are many studies support our finding at 2013 in China by Juanli Yuan et al[16]. Our study proved that about two-third of patients presented with GIT manifestations which include diarrhea, vomiting, weight loss, steatorrhea, but many patients presented non –GIT signs like short stature or pica and there many studies support our findings as study by Joseph A Murray et al 2004 and this support the thesis which suggest that CD is autoimmune disease and can affect many system like endocrine and GIT systems and may be present in association with other autoimmune diseases[17-19].

New guidelines on the diagnosis and treatment of celiac disease by the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition state that tTG-IgA testing should be used for follow-up care. Interpreting this test result is straightforward: a celiac pt. on the gluten-free diet for at least several months should have a negative test. The numerical value of the test is not important. Follow-up is very important because most patients with celiac disease get better symptomatically with therapy, they may not realize the need for follow-up. This view may be shared by some of their health care givers. Hence, not all patients with celiac disease get regular follow-up care. Follow-up is necessary not only to assess symptomatic recovery and to monitor complications but also to assist the patient in adhering to the diet. Follow-up care is the cornerstone to dietary compliance[15]. There is good objective clue that proactive follow up measures can support adherence to the gluten-free diet, both in children and adults[16]. Follow-up visits also provide a chance to give the patients updated information on new developments. Our

study clarified that antibodies measurements in CD patients are useful in clinical practice. Serial measurements of antibodies can allow objective targeting of dietetic resources. Our study is unique in that it highlights the benefits of measuring serial antibodies levels over several months in a routine clinical setting. This result is in agreement with previous work in[17] who showed a clear rise in antibodies concentration following a gluten challenge in patients with controlled CD. Similarly, the finding in this study that antibodies levels fall dramatically in the initial few months after starting GFD confirms the observations of previous studies[18,19]. And supports serial measurement every 3-6-9-12 months until the antibody level has normalized. The published literature on the use of serology in monitoring GFD is controversial and contradictory[20,21]. Whilst some studies have not found the rate of fall of antibody concentration to be a dependable marker of strict adherence to the GFD[22], others have found that normalized markers can be helpful to confirm on-going gluten–free dietary adherence. Some of the variability in findings may be interpreted by the use of the less specific guinea pig tTG substrate[23-25]. Moreover variability between studies may be caused by the different time courses of changes in serological markers, mucosal biopsy recovery, nutritional deficiencies and identifiable adherence to the GFD. The present study indicates how serial antibody measurements are able to provide unique informations with which to target dietetic resources. This study has demonstrated that complications of CD, particularly, are most commonly seen in those with persistently elevated antibodies[26,27].

Our study disagree with study done in Department of Pediatrics, Medical University Vienna, by Vécsei at 2013[17] this study demonstrated the limited value of serologic testing in the follow-up of pediatric CD with respect to the mucosal status. Only the normalization of EMA indicates mucosal healing with acceptable accuracy. Until more dependable tools are available for non-invasive follow-up, EMA should be used as follow up tool of first choice. However, more reliable noninvasive follow-up tools would be of great clinical and research usefulness with respect to the individualization of the GFD strictness and upcoming studies evaluating the efficacy of new CD treatment modalities, respectively[28]. Study done in Sweden and Supported by the Swedish Association against Asthma and Allergy which compared between tTga and EMA and stated that The human recombinant tTG-based ELISA is a sensitive, specific, and reproducible test to support the diagnosis and follow-up of childhood celiac disease and can be used as an alternative to the EMA test[24]. Our study is also supported by a study in France by Hosking[28], showed that deamidated gliadin antibodies are strongly related with VA and must be considered valuable tools in CD follow-up and that multiplex serologic analysis for treated CD represents a promising tool for personalized patient management[28].

References

1. Gujral N, Freeman HJ, Thomson ABR. Celiac disease: prevalence, diagnosis, pathogenesis and treatment. World J Gastroenterol 2012 (42):6036–59.
2. Cecilio LA, Bonatto MW. The prevalence of HLA DQ2 and DQ8 in patients with celiac disease, in family and in general population. Arq Bras Cir Dig. 2015 (3):183–5.
3. Parzanese I, Qehajaj D, Patrinicola F, Aralica M, Chiriva-Internati M, Stifter S, et al. Celiac disease: From pathophysiology to treatment. World J Gastrointest Pathophysiol. 2017 (2):27–38
4. Amil Dias J. Celiac Disease: What Do We Know in 2017? GE Port J Gastroenterol. 2017 (6):275–8.
5. Leonard MM, Sapone A, Catassi C, Fasano A. Celiac Disease and Nonceliac Gluten Sensitivity. JAMA 2017 (7):647.
6. Samasca G, Sur G, Lupan I, Deleanu D. Gluten-free diet and quality of life in celiac disease. Gastroenterol Hepatol from bed to bench 2014 (3):139–43
7. Rashid M, Lee J. Serologic testing in celiac disease: Practical guide for clinicians. Can Fam Physician 2016 (1):38–43.
8. Schyum AC, Rumessen JJ. Serological testing for celiac disease in adults. United Eur Gastroenterol J 2013 (5):319–25
9. Iacucci M, Ghosh S. Routine duodenal biopsies to diagnose celiac disease. Can J Gastroenterol. 2013 (7):385.
10. Sulkanen S, Halttunen T, Laurila K, Kolho KL, Korponay-Szabó IR, Sarnesto A, et al. Tissue transglutaminase autoantibody enzyme-linked immunosorbent assay in detecting celiac disease. Gastroenterology 1998 Dec (6):1322–8.
11. Joseph A Murray, Tureka Watson, Beverlee Clearman, and Frank Mitros. Effect of a gluten-free diet on gastrointestinal symptoms in celiac disease. Am J Clin Nutr 2004
12. Pietzak MM. Follow-up of patients with celiac disease: achieving compliance with treatment. Gastroenterology, 2005.
13. Lebwohl B, Granath F, Ekbom A, Smedby KE, Murray JA, Neugut AI, Green.70 PH, Ludvigsson JF: Mucosal healing and risk for lymphoproliferative malignancy in celiac disease: a population-based cohort study. Ann Intern Med 2013, 159(3):169–175.
14. Mehrdad, M., Mansour-Ghanaei, F., Frequency of Celiac Disease in Patients with Hypothyroidism, Hindawi Publishing Corporation, Volume 2012, Article ID 201538, 6 pages.
15. Anneli K. Carlsson, MD, Irene E.M. Axelsson, Serological Screening for Celiac in Healthy 2.5Year Old Children in Sweden2001.107:42-45
16. Juanli Yuan, Jinyan Gao, Xin LI, Fahui LIU, Cisca, The Tip of the Celiac Iceberg in China, 2013.
17. Vécsei E.: Follow-up of pediatric celiac disease value of antibodies in predicting mucosal healing, a prospective cohort study. BMC Gastroenterology 2014 14:28.
18. Midhagen G, Aberg AK, Olcen P, et al. Antibody levels in adult patients with coeliac disease during gluten free diet: a rapid initial decrease of clinical importance. J Intern Med 2004; 256: 519–24.
19. Bonamico M, Nenna R, Luparia RPL, et al. Radioimmunological detection of anti-transglutaminase autoantibodies in human saliva: a useful test to monitor coeliac disease follow-up. Aliment Pharmacol Ther 2008; 28: 364–70.

20. Vahedi K, Mascart F, Mary J, et al. Reliablity of antitransglutaminase antibodies as predictors of gluten free diet compliance in adult celiac disease. Am J Gastroenterol 2003; 98: 1079–87.

21. Leffler DA, Edwards George JB, Dennis M, A prospective comparative study of five measures of gluten-free diet adherence in adults with coeliac disease. Aliment Pharmacol Ther 2007; 26: 1227–35

22. Kaukinen K, Sulkanen S, Maki M, Collin P. IgA-class transglutaminase antibodiesin evaluating the efficacy of gluten–free diet in coeliac disease. Eur J Gastroenterol Hepatol 2002; 14: 311–5.

23. Haines ML, Anderson RP, Gibson PR. Systematic review: the evidence base for long-term management of coeliac disease. Aliment Pharmacol Ther 2008; 28: 1042–66

24. Sategna-Guidetti C, Pulitano R, Grosso S, et al. Serum IgA antiendomysium antibody titres as a marker of intestinal involvement and diet compliance in adult coeliac sprue. J Clin Gastroenterol 1993; 17:123–7.

25. Maki M, Collin P. Coeliac disease. Lancet 1997; 349: 1755–9.

26. Tursi A, Brandimarte G, Giorgetti GM. Lack of usefulness of anti-transglutaminase antibodies in assessing histological recovery after gluten free diet in celiac disease. J Clin Gastroenterol 2003; 37:387–91.

27. Tony Hansson, Ingrid Dahlbom, Recombinant Human Tissue Transglutaminase for Diagnosis and Follow-Up of Childhood Coeliac Disease. Pediatr Res, 2002

28. Hosking, F. Application of Deamidated Gliadin Antibodies in the Follow-Up of Treated Celiac Diseasepone. 2015.

Manuel Velasco (Venezuela) **Editor en Jefe** - Felipe Alberto Espino Comercialización y Producción
Reg Registrada en los siguientes índices y bases de datos:

WEB OF SCIENCE (WOS)
SCOPUS, EMBASE, Compendex, GEOBASE, EMBiology, Elsevier BIOBASE, FLUIDEX, World Textiles,
OPEN JOURNAL SYSTEMS (OJS),
REDALYC (Red de Revistas Científicas de América Latina, el Caribe, España y Portugal),
Google Scholar
LATINDEX (Sistema Regional de Información en Línea para Revistas Científicas de América Latina, el Caribe, España y Portugal)
LIVECS (Literatura Venezolana para la Ciencias de la Salud), LILACS (Literatura Latinoamericana y del Caribe en Ciencias de la Salud)
PERIÓDICA (Índices de Revistas Latinoamericanas en Ciencias), REVENCYT (Índice y Biblioteca Electrónica de Revistas Venezolanas de Ciencias y Tecnología)
SABER UCV, DRJI (Directory of Research Journal Indexing)
CLaCaLIA (Conocimiento Latinoamericano y Caribeño de Libre Acceso), EBSCO Publishing, PROQUEST

Esta Revista se publica bajo el auspicio del
Consejo de Desarrollo Científico y Humanístico
Universidad Central de Venezuela.

CDCH-UCV

cdch-ucv.net publicaciones@cdch-ucv.net

WWW.REVHIPERTENSION.COM
WWW.REVDIABETES.COM
WWW.REVSINDROME.COM
WWW.REVISTAAVFT.COM