Egyptian Journal of Medical Microbiology Volume 34 / No.4 / October 2025 45-50 Online ISSN: 2537-0979

ORIGINAL ARTICLE

Evaluation of serum miRNA21 and interleukin-15 in Celiac disease patients

Angham J.M. Ali, Ruaa M. Jaber*
Al-Furat Al-Awsat Technical University, Kufa, Iraq

Key words:
Assessment of IL-15 and MicroRNA21, Patients with Celiac Disease

Corresponding Author:
Ruaa Mohsen Jaber
Al-Furat Al-Awsat Technical University, Kufa, Iraq
mohsenruaa6@gmail.com

ABSTRACT

Background*: Celiac disease (CD), also known as "celiac sprue," is a persistent inflammatory condition that involves the small intestine, with an incidence of 1% in the majority of the population.* ***Objective:*** *This study aimed to investigate the role of interleukin-15 (IL-15) and MicroRNA21(miRNA21) among patients with Celiac Disease and comparing it with healthy controls.* ***Methodology:*** *This study was conducted on a total of (150) individuals in different sex and age group cases (40 males + 110 females) including (75) patients with Celiac Disease and (75) healthy individuals. CD patients were recruited at the Medicine Unit at Al-Sadder Medical City and the Specialized Hospital for Gastroenterology and Hepatology in Al-Najaf province. All patients were diagnosed with CD by serological and histological tests. The age range of the study population was from (3-45) years. Blood was drawn from a vein, to investigate microRNA21 by one-step qRT-PCR and IL-15 by ELISA technique.* ***Results:*** *The findings revealed that the mean of miRNA21 and IL-15 were elevated in CD patients compared to healthy people. Celiac Disease patients had a significantly higher mean of IL-15 than the healthy Group, 327.4 vs. 163, respectively (P.value <0.001). miRNA-21 expression was significantly upregulated in CD patients (fold change=6.21), compared to a control group (fold change=1.04). (P=< 0.001)* ***Conclusion****: The miR-21/IL-15 interaction may contribute to Celiac disease pathogenesis. Moreover, miR-21/IL-15 could be utilized as a biomarker to diagnose psoriasis and to assess its severity.*

INTRODUCTION

Celiac disease (CD) is a chronic, lifelong, multifactorial polygenic and autoimmune disorder, characteristically triggered by exposure to the exogenous factor “gluten” in genetically predisposed individuals, with resulting duodenal inflammation and enteropathy[1]. This results in symptoms that can manifest in various organs of the body, not just the gastrointestinal system[2]

CD can be classified as classic, non-classic, subclinical, silent, overt, potential, and refractory. The other way of classifying CD is based on location and histological appearance. Based on location, it can be categorized as intestinal vs extraintestinal or a combination of both[3]

Celiac disease can occur at any age from early childhood to old age. It has two peaks; the first peak occurs after gluten intake within the first 2 years of life, and the second is seen in the second or third decade of life[4].

Herrera-Quintana et al[5], reported that multiple factors play a role in the pathogenesis of CD, including environmental, immunological, and genetic factors. Consequently, diagnosis is based on a combination of criteria, including clinical, serological, genetic, and histological features. Early diagnosis is very important to prevent long-term complications[4].

MicroRNA (MiRNA) is one of the epigenetic factors that has a role in immunological dysregulation and inflammatory autoimmune disorders. It is associated with different miRNA expression levels, alterations in the targeted tissues and cells of either innate or adaptive immunity[6]

MicroRNAs (miRNA) are a group of small non-coding RNAs that regulate gene expression at the RNA level. MicroRNAs have positive regulatory effects on protein translation processes and often induce their performance by binding to the 3'-UTR mRNA region. Also, microRNAs are involved in various cellular processes, including development, cell division, cell signaling, and cell growth[7]

MiRNA-21 play key roles in the differentiation and function of the intestinal epithelium by regulating gene expression in both normal and pathological states, including inflammatory and autoimmune diseases [8]

MiRNAs can function as useful biomarkers but may also have distinct roles in CD pathophysiology through fine-tuning of gene expression levels. The cell types that play a key role in CD pathophysiology, e.g. intestinal epithelial cells, gluten-specific T cells, or intra-epithelial lymphocytes, selectively secrete or take up miRNAs

after the cells are stimulated with compounds that mimic the pathogenic conditions in CD [9].

The pro-inflammatory cytokine IL-15, recognized as a pivotal factor in the development of CD, is secreted by APCs and intestinal epithelial cells. It plays a central role in activating and promoting the proliferation of IELs, primarily CD8þ T cells, which attack the intestinal epithelium and contribute to villus atrophy [10]. It leads to the invigoration of intraepithelial lymphocytes, resulting in the CD's distinctive histologic changes. Interleukin-15 performs various biological functions critical for the retention and employment of various cell types; its up-regulation has been reported in many organ-specific autoimmune disorders, despite regulating its expression. The increase of IL-15 expression in the intestinal mucosa has become a telltale sign of CD, an intestinal inflammatory disorder caused by gluten intake [11].

IL-15 interacts with other cytokines to promote the maturation of dendritic cells, the proliferation of T and B cells, the cytotoxicity of NK and CD8+ T cells, and the production of proinflammatory cytokines, such as tumor necrosis factor (TNF)-α and IL-1β [12, 13]

The Aim of the present Study

To investigate the role of specific immune and genetic markers, particularly interleukin-15 (IL-15) and the gene expression of microRNA-21 (miRNA-21), in individuals with celiac disease, and to compare their levels with those in healthy individuals.

METHODOLOGY

Study design and population

This is a case-control study including 75 people with CD and the same number of healthy control subjects who volunteered. The patients and healthy groups were matched by age and sex. In every case, the celiac testing proved positive for serological tests at diagnosis time. Furthermore, a gastro-duodenoscopy was performed in all patients with CD and duodenal biopsy specimens taken from the second part of the duodenum. Exclusion Criteria in this study excluded patients aged less than 3 years or more than 45 years and also excluded patients who suffered from other autoimmune diseases as well as pregnant females.

Blood sampling and serum preparation:

Under complete aseptic conditions, 5 ml of blood was collected from every participant using a sterile, disposable plastic syringe. Then, the blood was collected into a vacutainer serum separator tube with polymer gel and clot activator for serum separation. After that, sera were separated into two aliquots; one of which was stored at -80 ^{0}C until measurement of the miRNA-21 level, and the other was stored at -20 ^{0}C until the IL-15 level was measured.

Measurement of serum IL-15 concentration:

Serum IL-15 concentrations were measured using an enzyme-linked immunosorbent assay (ELISA) kit (BT-Lab, China), according to the manufacturer's instructions. The final ELISA products were read using a microtiter plate reader (Human Diagnostics, Germany).

Measurement of serum concentration of MicroRNA-21 relative expression:

For assessment of miR-21, total RNA was extracted from serum using the RNA purification kits (TransZol™, TransGen, China) according to the manufacturer's instructions. Primers for miR-21 and U6 calibrators were designed by a biotechnology company (Macrogen, South Korea). U6 expression was used for the normalization of miRNA expression. The sequences of primers used for amplification of miR-21 and U6 are provided in Table 1. The RT-qPCR was carried out using an automated machine (RT-qPCR, Thermo Fisher Scientific, USA). The reaction was carried out using the master mix (1-Step RT-qPCR System, Promega, USA). The thermocycling conditions were: one cycle of reverse transcription at 37 ^{0}C for 15 minutes, then one Cycle for RT inactivation at 95 ^{0}C for 10 minutes, followed by 40 cycles each including denaturation at 95 ^{0}C for 10sec, then annealing at 60 ^{0}C for 30 sec and extension at 72 ^{0}C for 30sec.

Table 1: The sequence of primers that used in the present study

Primer	5`-3` Sequence
microRNA21	Forward 5`-GCCCGCTAGCTTATCAGACTGATG-3`
	Reverse 5-GTGCAGGGTCCGAGGT-3
U6	Forward 5`-GCGCGTCGTGAAGCGTTC-3`
	Reverse 5`-GTGCAGGGTCCGAGGT-3`

Gene expression (gene fold) value was calculated by the following equation:

Relative quantity (RQ) =2 (-ΔΔCT)

First, the average CT (cycle threshold) value for each triplicated sample was collected using an RT-PCR instrument to calculate the gene fold, and then ΔCT value was determined as follows for every sample:

Δ CT = CT (tested miR21) – CT (reference geneU6)

ΔΔCT=ΔCT (tested sample)-ΔCT (reference gene)

Fold gene expression RQ=2-(ΔΔCT)

Statistical Analysis

GraphPad Prism® software (Version 9.3.1) was used for statistical analysis. The difference in qualitative results among different groups was determined using the Chi-square test, Fisher's Exact Test and independent samples t-test. Results of IL-15 level and miR21 are presented as arithmetic mean ± standard deviation (±SD). One-way ANOVA was used for comparison between patients and the control group. P-values lower than 0.05 were considered statistically significant.

RESULTS

A total of 75 Celiac disease patients and 75 healthy controls were included in this case-control study. No statistically significant difference was observed between the two groups regarding sex and age (Table 2).

Serum level of miRNA21 relative expression

As explained in (Table 3), The average Ct value for miR-21 in the group of patients is 24.18, with a fold change of around 6.21 while the average Ct value for miRNA-21 in a control group is 24.03, with a fold change of around 1.04. Moreover, serum miRNA-21 concentrations were significantly higher in the patient's group than in the control subjects' group (P<0.001) as shown in Figure (1) and Table (4)

Table 2: Demographic data of the studied groups

Characteristic	Control *n* = 75	Celiac Disease *n* = 75	*P*
Age (years)			
Mean ±SD	١٩,٨ ± ١١	١٧,٨ ±١٠,١	0.٤ I NS
Range	٤,٠ – ٤٥	٣,٠ – 40	
Gender			
Male, *n* (%)	٣١ (٤١%)	٢٩ (٣٩ %)	0.٨ C NS
Female, *n* (%)	٤٤ (٥٩%)	٤٦ (61 %)	

n: number of cases; SD: standard deviation; Fisher's Exact Test.; I: independent samples t-test; NS: not significant (p ≥ 0.05).

Table 3: Calculating the gene expression of miRNA-21 with CD by RT PCR

Groups	*Means Ct of miRNA21*	*Means Ct of U6*	*ΔCt (Means Ct of miRNA21)*	$2^{-\Delta Ct}$	*experimental group/ Control group*	*Fold of gene expression*
Celiac Disease	**24.18**	**20.66**	**3.52**	**2.635**	**0.078972/ 0.4320**	**6.21**
Control	**24.03**	**17.87**	**6.16**	**0.4320**	**0.4320/ 0.4320**	**1.04**

Table 4 : Mean of fold miRNA21

Characteristic	Control *n* = 75	Celiac Disease *n* = 75	*P*
miRNA21			
Mean ±SD	1.04 ± 0.304	6.21 ± ١.43	<0.001 I***
Std. Error of Mean	0.05304	0.05304	

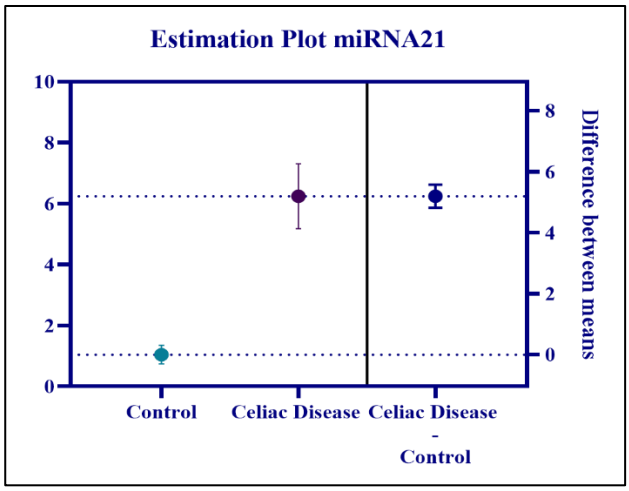

Fig. 1: Graph shows fold change in miRNA21 expression across groups.

Serum IL-15 level

Accessing the level of IL-15 concentration in the patient's serum with CD which proved the excess secretion of this cytokine, by which the patients group showed a higher level rather than in healthy controls, who did not show any elevation in cytokine concentration (P value <0.001), as shown in following table (5) and figure (2), which represented mean concentration of IL-15 in patients group as 327.4 ng/L, while in the control group was 163 ng/L.

Table 5 : Comparison of IL15 in Patients with Celiac Disease and control group

Characteristic	Control *n* = 75	Celiac Disease *n* = 75	*P*
Interleukin-15 (ng/ml)			
Mean ±SD	163 ± 36	327.4 ± 115	<0.001
Range	98.2–220.3	204.9–679.6	I***

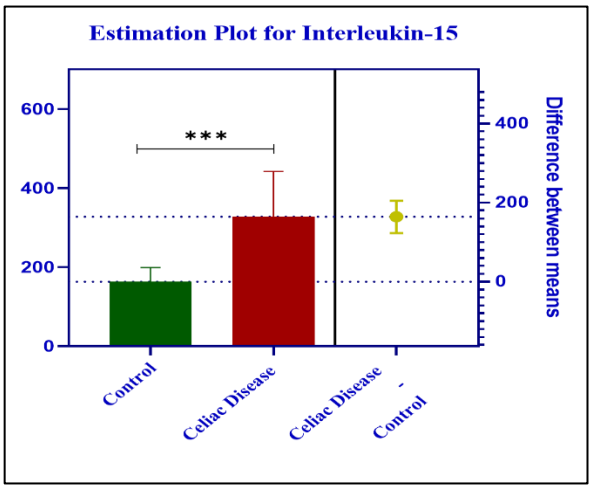

Fig. 2: Mean Serum IL-15 Concentration in patients and control

DISCUSSION

Celiac disease is a chronic autoimmune disorder of the small intestine triggered by gluten ingestion in genetically predisposed individuals [10]

Rigo et al[8] showed that the miRNA21 is involved in a cascade of immune mechanisms and consequences of inflammatory disorders in CD, such as cell proliferation and differentiation, regulatory T-cell development, innate immune response, activation of the inflammatory cascade, focal adhesion, T-cell commitment, tissue transglutaminase synthesis, and the cell cycle.

In CD patients, an increased expression of miR-21-5p was detected, possibly caused by a regulatory loop with its putative target STAT3. Therefore, this study demonstrated that miRNAs with altered expression in the duodenal mucosa differ between pediatric and adult CD patients[14].

Also, the overexpression of circulating miRNA21 in patients with untreated CD, and overexpression of circulating miRNA21 in the patients with tTG-IgA positive CD patients were determined [14].

In the study of Amr et al. [15], they examined the levels of miR- 21 in the blood serum. The researchers discovered that there was no notable difference in these levels between patients with CD who were following a gluten-free diet and the control group. However, they did observe that patients who were recently diagnosed with CeD had higher levels of miR-21 in their blood compared to the control group.

In a study conducted by Baldassarre et al[16], they have shown that the majority of research in the past has concentrated on the modulation and profiling of miRNAs, specifically examining in the intestinal mucosa of individuals with CD expression levels (referred to as tissue miRNAs). However, more recently, miRNAs have been discovered to circulate in various body fluids for example plasma as well as serum. These miRNAs are encoded by protein binding or enclosed in vesicles and secreted into outer space. Numerous researches have highlighted the significance as disease possible markers.

miR-21-5p was demonstrated to have a critical role in cytokine modulation, adaptive immune responses, colon epithelial cell hemostasis, as well as complications associated with IBD. Additionally, it has been shown that miR-21-5p increases intestinal permeability as a consequence of epithelial injury[17].

In the present study, Celiac patients demonstrated significantly higher serum concentrations of IL-15 than the control group.

This result is in the same line with another study [18] which reported that interleukin (IL)-15 is a proinflammatory cytokine, upregulation in CD induced by gluten peptides leads to disruption of intestinal

immune homeostasis, and a range of inflammatory consequences.

Also, Eidan and Mubark[19] found that Interleukin-15 is crucial in the development of Celiac disease. The increase in IL-15 expression in the lining of the intestines has become a characteristic feature of the CD.

IL-15 is a pro-inflammatory cytokine produced by intestinal epithelium and lamina propria of CD patients. Untreated CD patients had considerably increased IL-15 expression in the peripheral blood of CD patients compared to healthy controls.

Interleukin-15 performs various biological functions critical for the retention and employment of various cell types; its up-regulation has been reported in many organ-specific autoimmune disorders, despite regulating its expression. The increase of IL-15 expression in the intestinal mucosa has become a telltale sign of CD [20].

T cells overproduce cytokines when exposed to gluten, setting off an inflammatory response and revving up B-lymphocytes and the activation of autoimmune processes [21]

Also, Santonicola et al[10] proved that IL-15 in CD patients promotes the activation of IELs. These IELs express natural killer (NK) cell receptors, such as NKG2D, and target intestinal epithelial cells (IECs) that express its ligand, MICA, leading to their destruction. This cytotoxic activity exacerbates epithelial barrier dysfunction and enhances gluten peptide translocation.

IL-15 overexpression in IECs and adaptive anti-gluten immunity are required for CD8+ cytotoxic intraepithelial T cells (IE-CTLs) to mediate tissue destruction by acquiring a fully activated killer phenotype. Production of IL-15 by epithelial cells and innate immune cells inhibits intraepithelial lymphocyte (IEL) apoptosis, induces IEL to proliferate and release proinflammatory cytokines, and promotes perforin and granzyme-mediated cytotoxicity [22, 23].

CD patients with active, latent, or GFD express IL-15 differently. Increased levels of IL-15 in the intestinal mucosa were identified in patients with active CD in a previous investigation. Furthermore, the present finding that IL-15 levels were considerably more significant in individuals with untreated CD compared to the control group [21], in keeping with this hypothesis, potential CD patients, who conserve a normal intestinal morphology despite having lost oral tolerance to gluten lack IL-15 upregulation in IECs [22.]

Another study demonstrated that IL-15 gene expression is increased in biopsy specimens of CD patients with Marsh II compared with the control group, also found the overexpression of IL-15 in lamina propria and intestinal epithelium of patients with active CD [18, 23]

There are strong positive associations between IL-15 levels and the disease's histological severity. It is virtually nonexistent in the lamina propria and only occurs on villous enterocytes in healthy individuals. Conversely, enterocytes and lamina propria mononuclear cells enhance it when the body is in an inflammatory state [13].

CONCLUSION

There is a significant elevation in IL-15 and miRNA21in the patient's group other than in the control subjects group. and IL-15 and miRNA21 may contribute to the diseases or disorders that these people suffer from it.

Ethical consideration

The Department of Medical Laboratories/College of Health and Medical Technologies in Kufa (No. 5186) and the Training and Development Center in Najaf Health Directorate (No. 39053) all provided their approval for the current study. Additionally, all participants in both groups provided their informed written consent.

Acknowledgements

We would like to thank Medical Laboratories/College of Health and Medical Technologies in Kufa and the patients for their participation.

Competing interests

The author(s) declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

Finance

The research was funded by self-efforts.

REFERENCES

1. Aitella E, Cozzolino D, Ginaldi L, Romano C. Celiac Disease: A Transitional Point of View. Nutrients. 2025;17(2):234. doi:10.3390/nu17020234
2. Verma AK, Kumar S, Singh T, Mohan A, Sharma NR, Kumar A. Deciphering pH-Driven Dynamics of Prolyl Endopeptidases: Unveiling Structural insight in Celiac Disease using Computational Techniques. Published online January 1, 2025. doi:10.21203/rs.3.rs-5708047/v1.
3. Tarar ZI, Zafar MU, Farooq U, Basar O, Tahan V, Daglilar E. The Progression of Celiac Disease, Diagnostic Modalities, and Treatment Options. Journal of investigative medicine high impact case reports. 2021;9:23247096211053702. doi:10.1177/23247096211053702.
4. Sahin Y. Celiac disease in children: A review of the literature. World Journal of Clinical Pediatrics. 2021;10(4):53-71. doi:10.5409/WJCP.V10.I4.53.
5. Herrera-Quintana L, Navajas-Porras B, Vázquez-Lorente H, et al. Celiac Disease: Beyond Diet and Food Awareness. Foods. 2025;14(3):377. doi:10.3390/foods14030377.

6. Abaas BM, Darweesh MF. Profiles of miRNA expression and IFN-γ serum level as biomarker for the development of Multiple Sclerosis. Egypt J Immunol. 2024 Jul;31(3):62-70. PMID: 38995669
7. Kanwal N, Al Samarrai OR, Haider Al-Zaidi HM, Mirzaei A, Heidari MH. Comprehensive analysis of microRNA (miRNA) in cancer cells. Cellular, Molecular and Biomedical Reports. 2023;3(2):89-97. doi:10.55705/cmbr.2022.364591.1070.
8. Rigo FF, de Oliveira ECS, Quaglio AEV, Moutinho B, Di Stasi LC, Sassaki LY. Expression of MicroRNAs in Adults with Celiac Disease: A Narrative Review. International Journal of Molecular Sciences. 2024;25. doi:10.3390/ijms25179412.
9. Amr K, Bayoumi FS, Eissa E, Abu-Zekry M. Circulating microRNAs as potential non-invasive biomarkers in pediatric patients with celiac disease. European annals of allergy and clinical immunology. 2019;51(4):159-164. doi:10.23822/EURANNACI.1764-1489.90.
10. Santonicola A, Soldaini C, Ciacci C. New therapies in celiac disease. Current Opinion in Gastroenterology. Published online January 25, 2025. doi:10.1097/mog.0000000000001080.
11. Al-Omari RSM, Al-Ammar MH. Association between tnf-α (-308g →a) gene polymorphism and burn patient with sepsis (Open Access) (2021) International Journal of Drug Delivery Technology, 11 (1), pp. 217-221. doi: 10.25258/ijddt.11.1.41.
12. Al-Fatlawi, M.M.H., Al-Ammar, M.H., Al-Manssori, Y.L.H.Study of gene expression of Cytokine Genes (TLR-4, NOD-2) in patients with Otitis Media in Al-Najaf Governorate, Iraq (2024) BIO Web of Conferences, 84, art. no. 03019.https://www.bio-conferences.org/ Doi: 10.1051/bioconf/20248403019.
13. Jaber HY, Ali AJM. (2024, March). Evaluation of serum micro 155 in breast cancer. In AIP Conference Proceedings (Vol. 3092, No. 1). AIP Publishing.
14. Doğan G, Boyacioglu S, Caliskan M, Kasap E, Ayhan S, Kasirga E. Association of micro RNA expressions with pediatric celiac clinical findings. Genetika. 2023;55(1):277-288. doi:10.2298/gensr2301277d.
15. Amr K, Bayoumi FS, Eissa E, Abu-Zekry M. Circulating microRNAs as potential non-invasive biomarkers in pediatric patients with celiac disease. European annals of allergy and clinical immunology. 2019;51(4):159-164. doi:10.23822/EURANNACI.1764-1489.90.
16. Mousa MM, Ali AJ, Ben Romdhane WM. The Major role of TNF-α and miR-203 in the Immune Response of Diabetic Foot Ulcer. Egyptian Journal of Medical Microbiology, 2025; 34(1). DOI 10.21608/ejmm.2024.338511.1379.
17. Pelizzaro F, Cardin R, Sarasini G, et al. Crosstalk between microRNAs and Oxidative stress in coeliac disease. Inflammatory intestinal diseases. Published online January 24, 2024. doi:10.1159/000536107.
18. Khalkhal E, Rezaei-Tavirani M, Asri N, et al. Introducing New Potential Biomarkers for Celiac Disease among the Genes Extracted from General Databases. Middle East Journal of Digestive Diseases. 2022;14(2):192.
19. Jaber HY, Mohmmed Ali AJ (2024, March). Evaluation of p53 in women diagnosis with breast cancer. In AIP Conference Proceedings (Vol. 3092, No. 1). AIP PublishingAbadie V, Jabri B. IL-15: a central regulator of celiac disease immunopathology. Immunological Reviews. 2014;260(1):221-234. doi:10.1111/IMR.12191.
20. Alhammami, H.F., Al-Ammar, M.H.Study on correlation between il-33 serum level, il-33 gene single nucleotide polymorphism and rheumatoid arthritis susceptibility.(2020) Indian Journal of Forensic Medicine and Toxicology, 14 (3), pp. 955-960.http://www.ijfmt.com/issues.htm.
21. Mansor MR, Alammar MH. Evaluation of miRNA-155 expression in patients with Alzheimer's disease. Egyptian Journal of Medical Microbiology, 2024; 33(4). DOI: 10.21608/ejmm.2024.306484.1283.
22. Sedda S, Dinallo V, Marafini I, et al. mTOR sustains inflammatory response in celiac disease. Scientific Reports. 2020;10(1):10798. doi:10.1038/S41598-020-67889-4.
23. Majeed HA, Alammar MH. Immunomolecular Investigation Of Patients Infected With Ventilator Associated Pneumonia In Najaf Province (Open Access) (2019) Biochemical and Cellular Archives, 19 (2), pp. 4347-4350. doi: 10.35124/bca.2019.19.2.4347.