DOI: https://doi.org/10.53350/pjmhs22164475

ORIGINAL ARTICLE

Role of Autophagy Markers (Light Chain 3B and Beclin-1) in Pathogenicity of Breast Cancer in a Sample of Iraqi Women: A Pilot Study

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ABSTRACT

Background: Breast cancer (BC) is the most common malignancy in women and the second greatest cause of cancer death. There are relatively little data available on the role of autophagy markers in pathogenicity of breast cancer in the Iraqi population. We have therefore investigated the role of Baclin-1 and LC3-B in pathogenicity of breast cancer in Iraqi women.

Methods: A case-control study was conducted to assess the role of Bacline-1 and LC3-B in pathogenicity of breast cancer in Iraqi women. The study included 50 patients with BC age matched with 50 healthy individuals served as a control group. The Baclin-1 and LC3-B concentrations were determined by enzyme-linked immunosorbent assay (ELISA) technique.

Results: Body mass index (BMI) were significantly increase in patients with BC compared to the controls. Serum Baclin-1 and LC3-B levels in cases were significantly higher (P= 0.001) than those in controls. According to histological tumor characteristics. Grade 2 and stage IIA were significantly higher in BC patients and the tumor grade and stage did not show any significant associations with serum levels of Bacline-1 and LC3-B. No significant correlation between serum levels of Baclin-1 and LC3-B (r = 0.034; P = 0.812).

Conclusion: The study indicated that the Beclin-1 and LC3-B my consider a good biomarkers for progression of BC and it could be a therapeutic target to reduce the aggressiveness of disease.

Keywords: Breast cancer; Baclin-1; Light Chain3B.

INTRODUCTION

Breast cancer (BC) is defined as a malignant tumor that arises from the breast's ducts or lobules (Al-Abassi et al., 2018; Rajagopal et al., 2020). It is the most often diagnosed cancer in women, accounting for 11.6 % of newly diagnosed cancer cases and 6.6 % of estimated cancer mortality worldwide (Bray et al., 2018)

Between 2006 and 2012, the incidence of breast cancer in Iraq increased significantly, rising from 30 per 100000 to 40 per 100000 (Al-Saadi et al., 2021). In 2011, there were 3763 cases of breast cancer in Iraq, with an incidence rate of around 23.01 per 100000 females, compared to 16.65 per 100000 females in 2008 (Al-Musawi et al., 2017).

In Iraq, breast cancer was the most frequent malignancy, with 4529 cases reported in 2013, with 4422 females and 107 males, a proportion of the total of 18.84 % and a rate of 12.9 per 100000 people. As a result, breast cancer is the first of the top ten malignant neoplasms affecting the community (Almohaidi et al., 2021; Salman et al., 2021). In 2016, 897 women died as a result of this disease, which is the leading cause of cancer-related mortality among Iraqi females 23.6% and the second overall 12.1% among males and females after bronchogenic carcinoma (Alwan et al., 2019). The main characteristic of tumor cells is their disrupted adhesion to the ECM, which results in the loss of control over normal cell function. This attachment is important for signal transduction from the outside to the inside of the cell, which stimulates many activities such as cell cycle progression, and cells that separate from the ECM die via apoptosis (Al-Abassi et al., 2019)

Autophagy, a highly conserved intracellular degradation pathway, is required for cellular homeostasis to be maintained (Zhou et al., 2020). It is a complex biological process that's mostly controlled by autophagy-related genes (Atg). The yeast Saccharomyces cerevisiae has 32 Atg genes, and several homologs have been discovered in humans and other animals (He and Klionsky, 2009). Beclin-1 (encoded by BCN1, a human homolog of the yeast Atg 6 gene) and microtubule-associated light chain 3 (LC3, homolog of the yeast Atg 8 gene) are major mediators of autophagy (Hamurcu et al., 2019).

Beclin-1, the human or homology of yeast Atg 6, is important for autophagy initiation and control (Zhou et al., 2020). Beclin-1 is necessary for the development of autophagosomes (nucleation phase). When autophagy is initiated, LC3-A is lipidated to LC3-B, and subsequently LC3-II is localized on the autophagosomal membrane and plays a major role in autophagosomal membrane elongation. As a result, LC3-B has been widely used as a marker for autophagosome production and autophagy (Hamurcu et al., 2019 ; Abdulrazzaq et al., 2021).

Numerous studies have shown that autophagy is involved in a number of biological processes, including cell development and differentiation, cell death and survival, stress-induced adaptation, and immune responses (Cao and Klionsky, 2007; Kroemer and Levine, 2008; Kroemer et al., 2010). Autophagy dysfunction has been linked to a variety of diseases, including cancer, metabolic and neurological disorders, as well as cardiovascular and pulmonary diseases (Choi et al., 2013). Beclin-1 and LC3-B levels, as well as any relationship between beclin-1 and CL3-B and cancer outcome in humans, have not been well studied in our population.

We investigated the role of LC3-B and Beclin-1 in breast cancer in Iraqi women and correlated the concentration of these proteins with the clinical outcomes of the patients.

MATERIALS AND METHODS

Subject: Baghdad, Iraq's capital, receives thousands of visitors each day. The medical city's oncology teaching hospital in Baghdad is one of Iraq's largest centers, employing thousands of Iraqis from all around the country. As a result, the participants in the study may be representative of the Iraqi population. From November 2021 to January 2022, a case-control study was done in the biology department, college of education, university of Baghdad. This study involved 100 women who were separated into two groups: breast cancer women (n=50) as patients group and apparently healthy women (n=50) who served as controls.

Women diagnosed with breast cancer by specialist physicians. Mammography or histological findings confirmed the diagnosis of breast cancer. The study excluded cases that had other forms of cancer or were treated with mastectomy, chemotherapy, or radiotherapy.

A healthy control group was enrolled that did not have breast cancer, other types of cancer, or any history of acute or chronic disease (T2DM, liver disease, or autoimmune disease).

Questionnaires were used to ask all participants in the current study about their age, family history, medical history, and other diseases. The ethical committee at Baghdad University accepted the study procedure, and all participants completed a written informed consent document.

Blood samples: In a plain tube, all individuals' peripheral blood samples were collected. By using an enzyme linked immunosorbent assay (ELISA), autophagy markers such as

Baclin-1 and light chain 3B (LC3-B) were measured. The concentrations of circulating Baclin-1 and LC3-B in plasma were determined using a commercial ELISA kit (BT LAB, China) according to the manufacturer's instructions (Cat.No: MBS732891 and Cat.No: E4774Hu, respectively).

Statistical analysis: Statistical software SPSS (version 26) was employed to assess the significant differences in all investigations. Numbers and percentages were used to represent qualitative data. A mean and standard deviation were assigned to continuous variables. A student t-test was used for parametric variables to see if there were any differences between the two groups. For several mean comparisons within patient groups, the one-way ANOVA test was used. The association between variables was investigated using Pearson's correlation coefficients. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Clinical characteristics of participants: Table 1 summarizes the clinical features of patients and controls. Between patients and controls, there were significant differences in body mass index (BMI). Plasma Baclin-1 and LC3-B levels in cases [1.55± 0.44 ng/ml (mean ± SD) and 246.1± 36.3 ng/l (mean ± SD), respectively] were significantly (P= 0.001) higher than those in controls [0.79 ± 0.11 ng/ml (mean ± SD) and 132.4 ± 43.0 ng/l (mean ± SD), respectively].

Table 1: Clinical and laboratory characteristics of studied groups

Variable	Control (n=50)	BC (n=50)	P-Value
BMI (kg/m^2), (mean ± SD)	22.2 ± 1.8	26.9 ± 3.3	0.001
Age (years), n (mean ± SD)			
≥ 50	n= 16 (55.5± 4.2)	n= 25 (57.3 ± 7.1)	0.353
≤ 50	n= 34 (39.1± 6.0)	n= 25 (34.1 ± 4.9)	0.009
Family history (%)			
Yes	-	39 (78%)	-
No	-	11 (22%)	-
Autophagy Markers, (mean ± SD)			
Baclin-1 (ng/ml)	0.79 ± 0.11	1.55 ± 0.44	0.001
LC-3B (ng/l)	132.4 ± 43.0	246.1 ± 36.3	0.001

The values represent percentages or mean ± SD values. Significant p-values are shown in bold fonts. Abbreviations: n: number; SD: standard deviation; BC: breast cancer; BMI: Body mass index; LC-3B: Light chain3B.

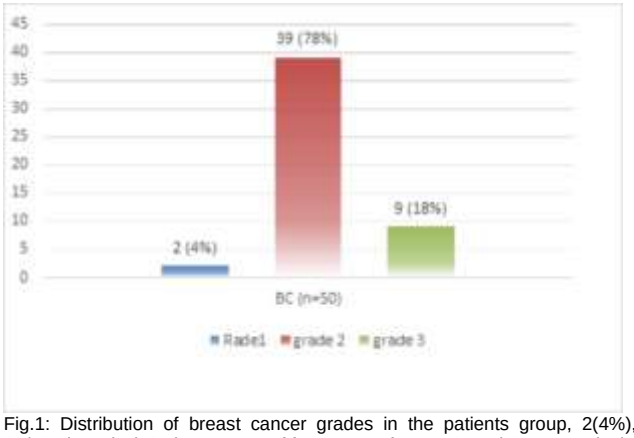

Fig.1: Distribution of breast cancer grades in the patients group, 2(4%), 39(78%), and 9(18%) represent of frequency of grae1,2, and 3 , respectively

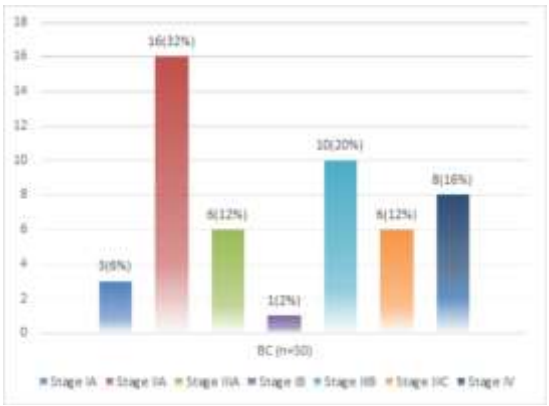

Fig.2: Frequencies of breast cancer stages in the patient group. Stage IIA is highly significant 16 (32%) compared with other stages

Table 2: The distribution of Age, Bacline-1, and LC3-B according to BC grades

BC (n=50)				
Parameter	Grade1 (n= 2)	Grade2 (n= 39)	Grade3 (n= 9)	P-Value
Age (Years), Mean ± SD	46.0± 8.4	50.5± 9.9	47.8±9.2	0.644
Bacline-1 (ng/ml), Mean ± SD	1.65 ± 0.79	1.55± 0.43	1.54± 0.50	0.955
LC-3B (ng/l), Mean ± SD	254.4 ± 89.0	247.1 ± 35.5	246.4 ± 31.8	0.720

The values represent mean ± SD values. Abbreviations: n: number; SD: standard deviation; BC: breast cancer; BMI: Body mass index; LC-3B: Light chain3B.

Table 3: The distribution of Age, Baclin-1, and LC3-B according to BC stages

BC (n=50)			
Stages	Age (Years), Mean ± SD	Bacline-1 (ng/ml), Mean ± SD	LC-3B (ng/l), Mean ± SD
IA (n=3)	45.3± 6.8	1.1± 0.07	226.0 ± 57.0
IIA (n=16)	52.1± 9.9	1.5± 0.4	238.7± 40.9
IIIA (n=6)	42.0± 5.3	1.8± 0.37	259.1± 20.0
IIB(n=10)	50.6± 6.8	1.6± 0.43	242.7± 42.2
IIIC (n=6)	49.3± 12.9	1.3± 0.45	274.6± 15.2
IV(n= 8)	53.2± 11.8	1.6± 0.41	246.9± 24.2
P-Value	0.345	0.138	0.291

The values represent mean ± SD values. Abbreviations: n: number; SD: standard deviation; BC: breast cancer; BMI: Body mass index; LC-3B: Light chain3B.

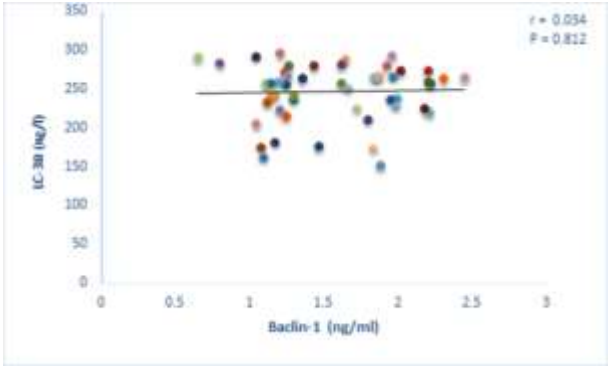

Fig.3: Distribution of Autophagy markers (Baclin-1 and LC3-B) in patient group, there were no significant correlation between Baclin-1 and LC3-B

Plasma Bacline-1 and LC3-B Levels and tumor characteristics: The distribution of BC women according to

histological tumor characteristics are shown in Fig.1 and Fig.2, respectively. Grade 2 and stage IIA were significantly higher frequent 39 (78%) and 16 (32%) in BC patients, respectively. Among the breast cancer patients, the tumor characteristics (grade and stage) did not show any significant associations with plasma levels of Baclin-1 and LC3-B as shown in Table2 and Table3.

Correlation between plasma Baclin-1 and LC3-B Levels: As shown in Fig.3, the correlation between plasma Baclin-1 and LC3-B Levels was studied in BC patients. No significant correlation was observed between the serum plasma Baclin-1 and LC3-B Levels ($r = 0.034$; $P = 0.812$).

DISCUSSION

In order to fully understand the mechanism responsible for developing of BC in the Iraqi population, as well as the clinical consequences associated with disease, this study focuses on the function of autophagy markers such as Becline-1 and LC3-B in pathogenesis of breast cancer in Iraqi women. Consequently, it may lead to more effective and supported diagnosis and treatment of BC patients.

Our current study showed the BMI which significantly higher in breast cancer compared with control. Increased BMI was associated to common cancers due to an increase in estrogen production in the adipose tissues (Arakaki et al., 2017). Our findings are consistent with previous studies (Abdul Kareem, 2018), Other studies, on the other hand, demonstrate a non-significant increase in BMI in BC women as compared to controls (Al-Saadi, 2021; Salman., 2021) .

In comparison to controls, women with breast cancer had higher levels of beclin1 and LC3-B, which is consistent with previous findings (Zhao et al., 2013; Choi et al., 2013). These findings support the hypothesis that beclin-1 and LC3-B are related to tumor suppression in human breast cancer tissue. To the best of our knowledge, no previous investigation on the role of LC3-B and Beclin-1 in women with breast cancer has been conducted in the Iraqi community. Autophagy's significance in cancer is currently being debated (Won et al., 2010).

Some scientists believe that autophagy promotes tumor growth, as evidenced by our data, whereas others believe that autophagy suppresses tumor growth and defends tumor cells from cell death stimuli (Won et al., 2010). Several mechanisms can be explored to describe how autophagy mediated by Beclin-1 and LC3-B allows cancer. First, there's autophagy, which causes cells to die. Second, we observed that Beclin-1 and LC3-B were significantly higher in breast cancer, which had never been seen in our community previously. The reverse Warburg effect, which describes the interaction of tumor and stromal metabolism in breast cancer, can explain the expression of autophagy-related proteins in the stroma. According to this theory, breast cancer cells produce reactive oxygen species that cause stromal glycolysis, mitochondrial malfunction, and enhanced autophagy. Ketone bodies and lactate produced by stromal cell glycolysis reach the cancer cell, which uses oxidative phosphorylation to generate ATP (Pavlides et al., 2009; Bonuccelli et al., 2010; Martinez-Outschoorn et al., 2010). As a result, according to this theory, greater autophagic activity in the stroma could explain the pathogenicity of Beclin-1 and LC3-B in the stroma. Several cell types produce inflammatory changes (Al-Abassi et al., 2015). During chronic inflammation, stromal cells have a function that extends beyond their usual function and is influenced by cytokines (Al-Abassi et al., 2015; Buckley, 2015).

CAFs are active fibroblasts in the cancer stroma that secrete cytokines and interact with the local extracellular matrix (BAI et al., 2022). Our results demonstrate no statistically significant association between Beclin-1 and LC3-B and grades of breast cancer in Iraqi women.

We observed that Beclin-1 level was no significant correlated with LC3-B in breast cancer tissue ($P = 0.812$), these findings are inconsistent with recent reports which have shown that Beclin-1 negatively regulates autophagic cell death (Pattingre et al., 2005). According to our results, we can conclude that Beclin-1 and LC3-B my consider a good biomarkers for progression of BC and it could be a therapeutic target to reduce the aggressiveness of disease.

Abbreviation list:

BC: Breast cancer
LC3-B: Light chain3B
ELISA: Enzyme linked immunosorbent assay
BECN1: Beclin-1 gene
T2DM: Type 2 diabetes mellitus
SD: standard deviation
ANOVA: Analysis of variance
MAP1LC3: microtubule-associated proteins 1A/1B light chain 3B
CAFs: cancer -associated fibroblast

Competing interests: The authors declare that they have no competing interests.

Authors' contributions: Fieldwork was done by Aamal Awda Al-Mussawi And Hazma Mossa Alabassi . Aamal Awda Al-Mussawi performed statistical analyses. Hazma Mossa Alabassi supervised the study and Aamal Awda Al-Mussawi drafted the manuscript under his supervision. The final manuscript was read and accepted by all authors.

Acknowledgments: The authors would like to express their thanks to the staff of the oncology teaching hospital of medical city (Baghdad, Iraq) for their assistance in collecting patient blood samples. Furthermore, they wish for the well-being of all patients.

REFERENCES

1. Abdulrazzaq, A. A., & Alabassi, H. M. (2021). DETECTION OF ANTIOXIDANT ACTIVITY AND ANTIBACTERIAL ACTIVITY OF DIFFERENT SAPONINS EXTRACT AGAINST CLINICAL PATHOGENS.
2. AL-ABASSI, Hazima Mossa; ALMOHAIDI, Asmaa M. Salih; ALMUSAWI, Amenah Ali Raheem. Determination of Integrinα2 (ITGA2), Progesterone, prolactin, estradiol, zinc and Vitamin C in serum of female Iraqi patients with breast cancer. Ibn AL-Haitham Journal For Pure and Applied Science, 2018, 73-86.
3. AL-ABASSI, Hazima M., et al. Serum profile of cytokines in Iraqi inflammatory bowel disease patients. Mustansiriya Medical Journal, 2015, 14.2: 11.
4. AL-ABASSI, Hazima M.,ASSESSMENT OF ZYXIN AND E-CADHERIN IN IRAQI PATIENTS WITH GLOMA OF THE BRAIN .Bchem.Cell.Arch,2019;19(2):4379-4383.
5. ALABASSI, Hazima Mossa K., et al. Comparative Study of Multiple Sclerosis Therapy by Interferon-β and Methylprednisolone of Iraqi Patients via Adenosine Deaminase Activity.
6. Abdulkareem, Z. J. (2018). Immunological and Molecular Study of IL-33 and ST2 Receptor in a Sample of Iraqi Patients with Breast Cancer. thesis M. sc. College of Education for Pure Science (Ibn AlHaitham) , University of Baghdad, Baghdad, Iraq. 133.
7. ARAKAKI, M., et al. Abstract P5-13-18: The feasibility of nurse intervention to improve and maintain the BMI of breast cancer survivors over 25 to appropriate levels. 2017.
8. AL-SAADI, Hamed Hussein Ali; AL-ABASSI, Hazima Mossa; JAWAD, Mohammed Mahdi. Role of Inhibitory Check Point (CTLA-4) in Iraqi Women with Breast Cancer. Indian Journal of Forensic Medicine & Toxicology, 2021, 15.3: 2337.
9. Al-saadi, H.H.A(2021). Immunogenetic study of Cytotoxic T-lymphocyte Antigen-4(CTLA-4)and Interleukin-10(IL-10)level of Iraqi women with breast cancer .thesis M.sc. College of Education for pure science(Ibn Al-Haitham),University of Baghdad,Baghdad,Iraq.108.
10. ALMOHAIDI, Asmaa M. Salih; AL-ABASSI, Hazima Mossa; ABDULKAREEM, Zainab Jalil. Sera Level and Polymorphism of Interleukin-33 Gene in Iraqi Females Patients with Breast Cancer. Ibn AL-Haitham Journal For Pure and Applied Sciences, 2021, 34.1: 1-11.
11. AL-MUSAWI, Amenah Ali Raheem; ALMOHAIDI, Asmaa M. Salih; AL-ABASSI, Hazima Mossa. The Role of Integrin α2 (ITGA2) factor in Iraqi Females Patients with Breast Cancer.
12. Bonuccelli G, Tsirigos A, Whitaker-Menezes D, Pavlides S, Pestell R, Chiavarina B et al. Ketones and lactate "fuel" tumor growth and metastasis. Cell Cycle. 2010;9(17):3506-3514.
13. BUCKLEY, Christopher D., et al. Stromal cells in chronic inflammation and tertiary lymphoid organ formation. Annual review of immunology, 2015, 33: 715-745.

14. BAI, Jingchao, et al. Targeting TNFR2 in cancer: all roads lead to Rome. Frontiers in Immunology, 460.
15. Cao Y, Klionsky D. Physiological functions of Atg6/Beclin 1: a unique autophagy-related protein. Cell Research. 2007;17(10):839-849.
16. Choi A, Ryter S, Levine B. Autophagy in Human Health and Disease. New England Journal of Medicine. 2013;368(7):651-662.
17. Choi J, Jung W, Koo J. Expression of autophagy-related markers beclin-1, light chain 3A, light chain 3B and p62 according to the molecular subtype of breast cancer. Histopathology. 2012;62(2):275-286.
18. Demographic and Clinicohistological Profiles of Women Diagnosed with Breast Cancer at Al-El Wiya Maternity Teaching Hospital / Baghdad: A Retrospective Study. Indian Journal of Forensic Medicine & Toxicology. 2021.
19. Erratum: Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal for Clinicians. 2020;70(4):313-313.
20. Hamurcu Z, Delibaşı N, Nalbantoglu U, Sener E, Nurdinov N, Tascı B et al. FOXM1 plays a role in autophagy by transcriptionally regulating Beclin-1 and LC3 genes in human triple-negative breast cancer cells. Journal of Molecular Medicine. 2019;97(4):491-508.
21. He C, Klionsky D. Regulation Mechanisms and Signaling Pathways of Autophagy. Annual Review of Genetics. 2009;43(1):67-93.
22. Kroemer G, Levine B. Autophagic cell death: the story of a misnomer. Nature Reviews Molecular Cell Biology. 2008;9(12):1004-1010.
23. Kroemer G, Mariño G, Levine B. Autophagy and the Integrated Stress Response. Molecular Cell. 2010;40(2):280-293.
24. Martinez-Outschoorn U, Balliet R, Rivadeneira D, Chiavarina B, Pavlides S, Wang C et al. Oxidative stress in cancer associated fibroblasts drives tumor-stroma co-evolution. Cell Cycle. 2010;9(16):3276-3296.
25. Pattingre S, Tassa A, Qu X, Garuti R, Liang X, Mizushima N et al. Bcl-2 Antiapoptotic Proteins Inhibit Beclin 1-Dependent Autophagy. Cell. 2005;122(6):927-939.
26. Pavlides S, Whitaker-Menezes D, Castello-Cros R, Flomenberg N, Witkiewicz A, Frank P et al. The reverse Warburg effect: Aerobic glycolysis in cancer associated fibroblasts and the tumor stroma. Cell Cycle. 2009;8(23):3984-4001.
27. Rajagopal T, Seshachalam A, Akshaya R, Rathnam K, Talluri S, Jothi A et al. Association of HOTAIR (rs920778 and rs1899663) and NME1 (rs16949649 and rs2302254) gene polymorphisms with breast cancer risk in India. Gene. 2020;762:145033.
28. SALMAN, Adel Omran; ALABASSI, HazimaMossa; MAHOD, WafaaSabri. Demographic and Clinico-Pathological Characteristics of Some Iraqi Female Patients Newly Diagnosed with Breast Cancer. Annals of the Romanian Society for Cell Biology, 2021, 25.6: 8264-8278.
29. Won K, Kim G, Kim Y, Song J, Lim S. Clinicopathologic correlation of beclin-1 and bcl-2 expression in human breast cancer. Human Pathology. 2010;41(1):107-112.
30. Zhao H, Yang M, Zhao J, Wang J, Zhang Y, Zhang Q. High expression of LC3B is associated with progression and poor outcome in triple-negative breast cancer. Medical Oncology. 2013;30(1).
31. Zhou Y, Liu K, Li X, Qin Y, Zhang Y, Zhang Y et al. Molluscan Beclin-1 is involved in the innate immune response by regulating the autophagosomes formation in Crassostrea hongkongensis. Aquaculture Reports. 2020;18:100541.