Original Research Article: Comparison of Exons 2 and 3 of DIRAS3 Gene in Mastectomies and Lumpectomies Women

Parmiss Adyani Kalvanagh[1] | Yousef Adyani Kalvanagh*[2]

[1]*Postgraduate Student, Experimental sciences, Tabriz, Iran*
[2]*Breast Surgery Fellowship, Tabriz University of Medical Sciences, Tabriz, Iran*

Use yor device to scan and read the article online

Citation P. Adyani Kalvanagh, Y. Adyani Kalvanagh*, **Comparison of Exons 2 and 3 of DIRAS3 Gene in Mastectomies and Lumpectomies Women.** *Eurasian J. Sci. Tech.*, ., **2023**, *3*(3), 154-164.

https://doi.org/10.48309/EJST.2023.392686.1080

Article info:
Received: 2023-03-11
Accepted: 2023-04-24
Available Online: 2023-05-25
ID: EJST-2304-1080
Checked for Plagiarism: Yes
Language Editor:
Dr. Behrouz Jamalvandi
Editor-in-Chef:
Professor Dr. Ali Nokhodchi

Keywords:
DIRAS3, Exons 2, Exons 3, Lumpectomy, Mastectomy

ABSTRACT

Introduction: According to researchers' research on the relationship between breast cancer and diabetes in the presence of other genes such as INSR, the aim of this study was comparing exons 2 and 3 of the DIRAS3 gene in mastectomies and lumpectomies women.
Procedure: DNA extraction was done using a kit (PZP Molecular IVD Company) and stored in a micro tube at -20C. The said kit contained four different buffers named Wash buffer (W), Binding buffer (B), Elution buffer (E) and Removal buffer (R) along with proteinase k powder.
Results: The polymorphism found in intron 2 with rs6682360 at position c.332-3 T>C or g.831 T>C of a cancer patient sample, that is, 3 nucleotides upstream of the 332nd nucleotide in intron 2 or the 831st nucleotide in intron 2 which is T open to C open. Since the number of measured samples was small, no significant relationship was found between the development of breast cancer and diabetes and the presence of this polymorphism.
Conclusion: The results of this research showed that among the available polymorphisms, only the 5'UTR c.331 G>A is related to breast cancer in mastectomies and lumpectomies women.

Introduction

In people who have breast cancer and their treatment is long, there is a possibility of developing diabetes, even in people who already had diabetes, this disease will increase with breast cancer. In addition, statistics have shown that people who have diabetes have the possibility of getting breast cancer and about 20% of these people get breast cancer [1-3]. Estrogen resistance in the stages of chemotherapy in people with breast cancer is considered as one of the causes of diabetes in these people. The use of some drugs such as glucocorticoid in chemotherapy increases a person's blood sugar. The use of these drugs is to prevent inflammation and nausea [4-6].
When the body becomes resistant to insulin, it becomes susceptible to the spread of diabetes

***Corresponding Author: Yousef Adyani Kalvanagh** (Adyani.Yousef.Kalvanagh@Gmail.Com)

and various cancers, which may occur in people who have breast cancer. Moreover, in diabetic people, with the increase of insulin level, breast tissue undergoes changes, which increases the risk of breast cancer [7]. Statistics have shown that people who have advanced breast cancer and have diabetes at the same time have larger tumors than other people with breast cancer, and in these people, the possibility of spreading the disease (both diabetes and cancer) is more [8-10].

The elimination of NOEY2 gene expression is directly related to the increased risk of ovarian and breast cancer, and in 41% of breast and ovarian cancers, the protein transcribed by it is not produced, and it is considered as a tumor suppressor gene. Therefore, in people who inherit both maternal chromosomes, this gene is not expressed and the person is exposed to the risk of breast and ovarian cancer [11-13].

As mentioned earlier, the DIRAS3 NOEY2//ARHI gene is probably a tumor suppressor gene. To clarify the clinical significance of mRNA of this gene in breast cancer, it was investigated that inactivation of this gene causes breast cancer tumors. As a result of analyzing the expression of NOEY2/ARHI gene using real time PCR technique, expression took place in all non-cancerous breast tissue samples, but in almost half of the cancerous tissue samples, NOEY2/ARHI gene was not expressed or its expression was significantly reduced [14-16].

In this research, the aim is to investigate the following parameters. Given that the DIRAS3 gene has been proven as a tumor suppressor candidate, can it play a role as a marker gene in breast cancer patients in the studied subjects? In addition, according to authors' research on the relationship between breast cancer and diabetes in the presence of other genes such as INSR, are there any mutations or polymorphisms in exon 2, intron 2, and exon 3 of the DIRAS3 gene?

Female condoms

Female condoms are used to protect women against STDs and HIV, as well as pregnancy. The female condom consists of a cylindrical tube made of polyurethane, one end of which is blocked by a closed loop that covers the cervix (Figure 1), and the other end of which has an open loop that covers the perineum [17-19].

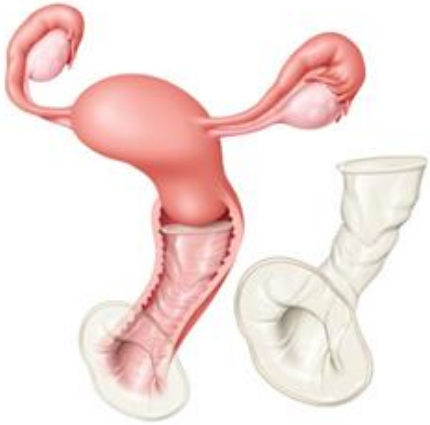

Figure 1. Female condom

Volvo vaginal infections

The vaginal area in the female reproductive system is protected against germs due to its low pH (3.5-4.5) due to the activity of two-line bacillus in the vagina. This bacterium suppresses the growth of anaerobic bacteria and produces lactic acid, which creates an acidic PH. Doderline bacilli also produce hydrogen oxide (Figure 2), which is toxic to anaerobic microbes [20-22].

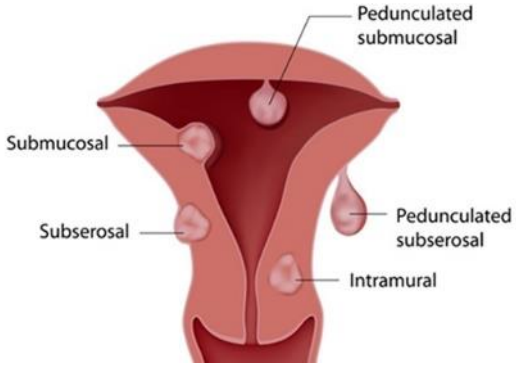

Figure 2. Vaginal Infections

Anaerobic bacteria in the vagina and Gardenela vaginalis (normal vaginal flora) and as well as the lack of lactobacilli occur due to overgrowth of bacteria (Figures 3 and 4) [23].

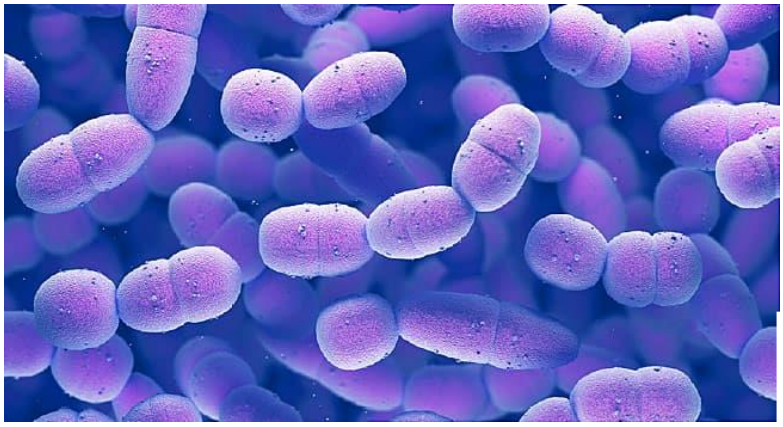

Figure 3. Incremental bacterial and viral infection

Metronidazole is an effective drug in the Trichomonas treatment. Both couples are given a single high dose or a lower dose three times a day for 1 week. While taking this medicine, some patients may feel a metal-like taste in their mouth (Figure 5). They will also experience nausea, vomiting, and hot flashes if they drink alcohol [24-26].

Figure 4. Trichomonas

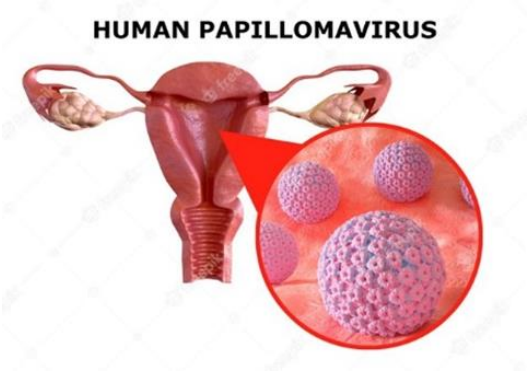

Figure 5. Human papillomavirus HPV sexually transmitted infection

Types 6 and 11 are the most common types of these viruses and cause wart-like growths germ can spread to the top (Figures 6, 7, and 8) (uterus and fallopian tubes of the pelvic cavity) [27-29].

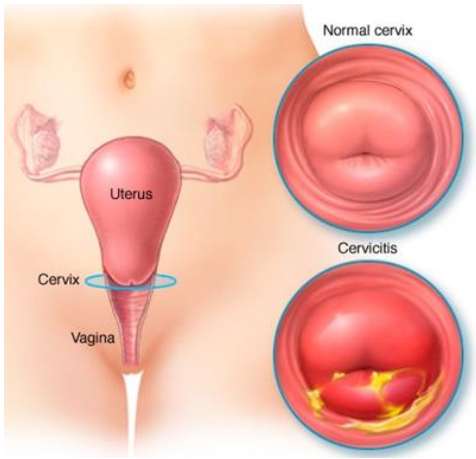

Figure 6. Cervicitis

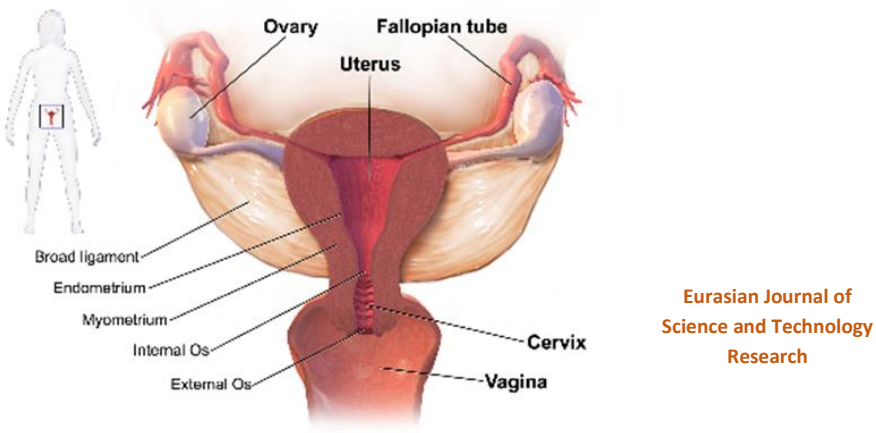

Figure 7. Pelvic inflammatory disease

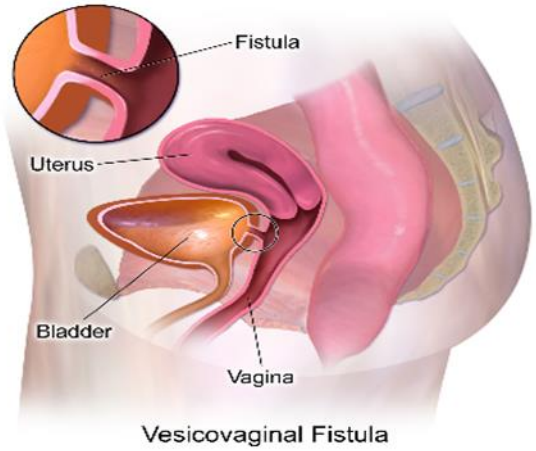

Figure 8. Vesicovaginal Fistula

a few years and when the atrophy of the genital tract occurs due to aging during menopause (Figures 9 and 10).

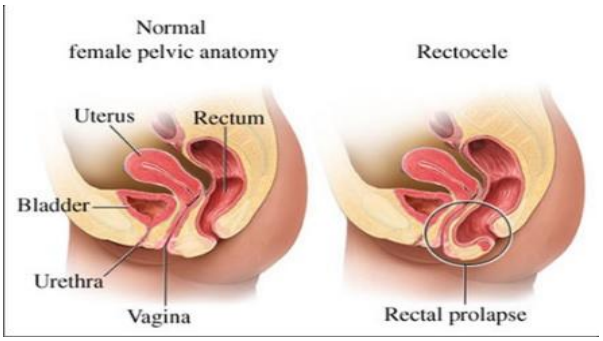

Figure 9. Pelvic organ prolapses

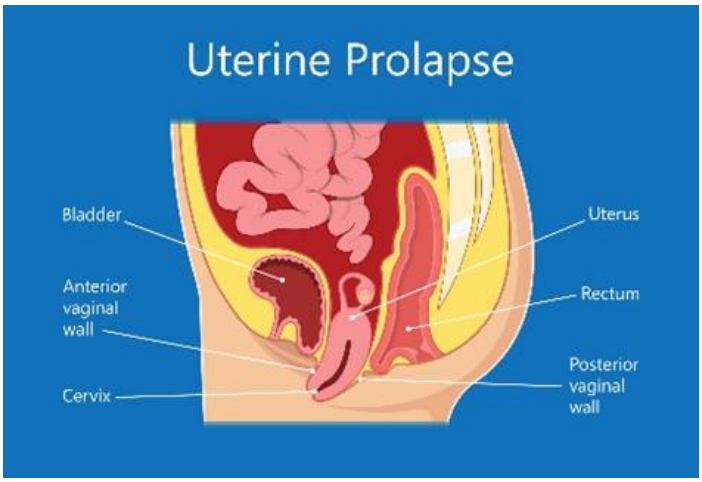

Figure 10. Uterine prolapse scaled

Method

In general, 50 women patients with breast cancer, breast cancer-diabetes and diabetes were selected as the case group and 50 healthy women who had no family history of breast cancer and other neoplastic symptoms, and also had no history of diabetes were selected as the control group.

In the stage of obtaining blood from the patients, after coordination with the laboratory, it was done in such a way that after receiving written consent from each of the studied patients and negotiating with them to explain the topic of the research, sampling from female patients was done after each stage of identifying the female patients. It was done gradually by the laboratory personnel, while it was explained to them that the results of the research will be made available to everyone in the form of a report after the laboratory procedures and the summary of the information obtained, and the patients' information will remain completely confidential.

3 ml of venous blood was taken from each patient and healthy person to extract DNA. DNA extraction was done using a kit (PZP Molecular IVD Company) and stored in a micro tube at -20C. The mentioned kit contained four different buffers named Wash buffer (W), Binding buffer (B), Elution buffer (E), and Removal buffer (R) along with proteinase k powder. The extraction steps were performed according to the mentioned protocol and the desired fragment was amplified by PCR-Sequencing technique. The materials used in preparing the PCR mix of the samples were prepared according to the concentrations mentioned in Table 2 with a total volume of 25 microliters. After PCR, the products were stored in the freezer for electrophoresis and sequencing. The PCR product was electrophoresed using 1.5% agarose gel and the efficiency of amplification reaction was observed using a gel imaging device (Gel doc). Thereafter, the samples with suitable band were qualitatively and quantitatively sequenced. It should be noted that in addition to the research samples, the positive control and negative control samples were subjected to PCR along with other samples, and then electrophoresed in 1.5% agarose gel. The sample used in the positive control was the sample in which the desired fragment was detected and the appropriate band was seen.

To determine the sequencing of PCR products, the products were sent to "Techazma" company located in Tehran. Next, the sequenced data were analyzed using Chromas and Gene Runner software. Chi-square, Odds Ratio, and P-value tests were used to compare the frequency distribution of three different genotypes in the found position (rs72668850 of DIRAS3 gene) in patient samples with the frequency distribution of these three genotypes in control samples.

Results

After performing the PCR reaction and observing the appropriate band, the products were sequenced. The sequenced fragments were read and analyzed using Chromas and Gene Runner software. 100 samples from people with diabetes, diabetes-breast cancer, breast cancer, and healthy individuals were sequenced. After determining the sequence of PCR products from the number of 50 healthy samples, 5 people were homozygous and 5 people were heterozygous, and from the number of 50 patient samples, 15 people with invasive ductal breast cancer (IDC) were heterozygous and 5 people were homozygous with one type of polymorphism in exon 3 in the 5'UTR position c.331 were G>A. Likewise, a cancer patient had a heterozygous polymorphism in intron 2 at position c.332-3 T>C, but no polymorphism was found in exon 2.

PCR products were kept in refrigerator for sequencing and loading in agarose gel. Figure 1-A (position 250) is related to the polymorphism with rs6682360, at position c.332-3 T>C, or g.831 T>C of the DIRAS3 gene is heterozygous, where the nucleotide T in intron 2 of the cancer patient sample has changed to C. Figure 1-B also shows the result of electro pherogram analysis of a cancer patient sample after sequencing. In this figure (position 261), the polymorphism was found in the 5'UTR c.331 G>A position. In other words, in the 331st nucleotide of exon 3, the base G has been changed to the base A, and as can be seen from the graph, the polymorphism found is heterozygous (Figure 11).

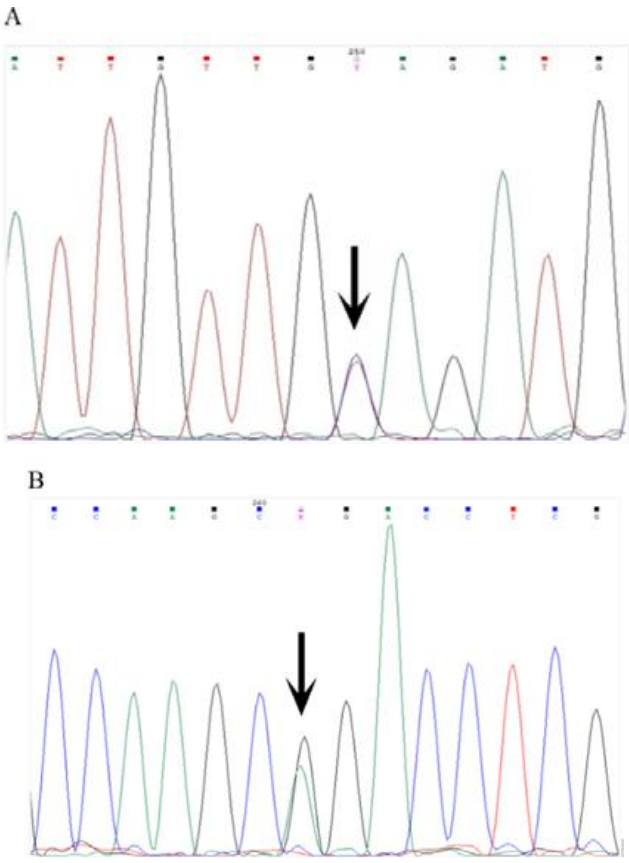

Figure 11. rs6682360polymorphism. (A) position 250 and (B) position 261

In the group of patients, 30 people (60%) have homozygous genotype G/G, 15 people (30%) have heterozygous genotype G/A, and 5 people (10%) have homozygous genotype A/A. In addition, in the control group, G/G genotype was observed in 40 people (80%), G/A genotype in 5 people (10%), and A/A genotype in 5 people (10%). Given that the obtained P (P=0.04) is smaller than 0.05, as a result, it shows a significant difference between two groups of cancer patients and controls for the G>A polymorphism with rs72668850 and a significant relationship between the polymorphism in exon 3 of the gene. There is DIRAS3 and breast cancer. As it can be seen, the obtained p-value (P=0.07) is greater than 0.05, but there is still a significant relationship between polymorphism in exon 3 of the DIRAS3 gene and breast cancer. This is because the alleles are generally measured relative to each other and do not show much difference in terms of balance and pathogenicity in the population.

The polymorphism found in intron 2 with rs6682360 at position c.332-3 T>C or g.831 T>C of a cancer patient sample, that is, 3 nucleotides upstream of the 332nd nucleotide in intron 2 or the 831st nucleotide in intron 2, which is T has been converted to open C. Since the number of measured samples was small, no significant relationship was found between the occurrence of breast cancer and diabetes and the existence of this polymorphism.

Discussion

Today, it has been found in various researches that diabetes mellitus increases the risk of many cancers, including breast, liver, pancreas, colon, endometrium, kidney, and bladder cancer. In addition, most diseases related between diabetes and cancers are related to insulin resistance, decreased fat control, oxidative stress, and changes in the immune system [30-32]. Furthermore, continuous use of diabetes medications and their supplements are apparently involved in cancers [33].

Breast cancer is significantly associated with a high level of insulin resistance in premenopausal overweight women or premenopausal obese women, but not in premenopausal women with normal weight or postmenopausal women with normal weight [34-36].

The risk of colon cancer in diabetic people has increased by 26% and the risk of dying from this type of cancer has increased by 30% compared to non-diabetic people [37-39].

In the investigation of polymorphisms in the insulin receptor gene (INSR) and its relationship with PCOS, diabetes and breast cancer among Iranian women, the results showed that there is no relationship between them [40-42].

In an experiment, the researchers found that the "promoter" and the "exon two region" of the NOEY2 gene are hot spots for mutations in this gene, and mutations that occur in the coding region of exon 2 and part of the promoter may change the NOEY2 expression [43].

First, they extracted and sequenced the DNA of 100 samples (50 pieces of breast cancer tissue and adjacent breast tissue along with 50 pieces of benign breast lesions). After data analysis, 21 of 50 (42%) mutations in breast cancer tissue (in the promoter, 11 in exon 2, 7 in the untranslated region, and 3 in the coding region) and 17 of 50 (34%) mutations were detected in adjacent breast tissues (6 cases in the promoter and in exon 2, 10 cases in the untranslated region, and 1 case in the coding region), and no mutations were detected in benign breast tissues.

In another study similar to the present study, researchers in an experiment entitled: "Screening for the presence of germline exon 1 polymorphism of the NOEY2 gene in women with polycystic ovary syndrome and diabetes in Iran using the PCR-Sequencing technique", examined exon 1 of the NOEY2 gene in patients with diabetes, diabetes measured polycystic, polycystic, and healthy patients and observed the mutation only in a patient with diabetes and found only polymorphism in other patients with diabetes, polycystic, and healthy [45].

Conclusion

It has been proven in various studies that mutations and polymorphisms of the DIRAS3 gene are involved in the development of breast cancer and ovarian cancer. In this study, the examined samples were 50 healthy people and 50 patients. Among the patients, no polymorphism was observed in the exon 2 sequence of the DIRAS3 gene, while among all samples, a polymorphism was found in intron 2 in a cancer patient at c.332-3 T>C, or g.831 T>C, and a type of polymorphism in exon 3 (both in cancer patients and controls) was observed in the 5'UTR c.331 G>A position.

ORCID

Parmiss Adyani Kalvanagh:
https://orcid.org/0000-0003-0143-5260
Yousef Adyani Kalvanagh:
https://orcid.org/0000-0001-6071-7626

References

[1] M.H. Abdollahi, K. Foruzan-Nia, M. Behjati, B. Bagheri, M. Khanbabayi-Gol, S. Dareshiri, A. Pishgahi, R. Zarezadeh, N. Lotfi-Naghsh, A. Lotfi-Naghsh, M. Naghavi-Behzad, *Nigerian medical journal: journal of the Nigeria Medical Association*, **2014,** *55,* 379-83. **[crossref], [Google Scholar], [Publisher]**

[2] M. Khanbabayi-Gol, F. Jabarzade, V. Zamanzadeh, *Nurs Midwifery Journal,* **2017**, *15,* 612-9. **[crossref], [Google Scholar], [Publisher]**

[3] M. Haghdoost, S. Mousavi, M.K. Gol, M. Montazer, *International Journal of Women's Health and Reproduction Sciences*, **2019,** *7,* 526-530. **[crossref], [Google Scholar], [Publisher]**

[4] R. Eghdam-Zamiri, M. Khanbabayi Gol, *The Iranian Journal of Obstetrics, Gynecology and Infertility*, 2020, *22,* 15-21. **[crossref], [Google Scholar], [Publisher]**

[5] N. Mobaraki-Asl, Z. Ghavami, M. Khanbabayi Gol, *Journal of education and health promotion*, 2019, *8*. **[crossref], [Google Scholar], [Publisher]**

[6] M. Khanbabayi-Gol, A. Dorosti, M. Montazer, *Journal of education and health promotion*, 2018, *8*. **[crossref], [Google Scholar], [Publisher]**
[7] N. Shahidi, F. Mahdavi, M. Khanbabayi-Gol, *Journal of Education and Health Promotion,* **2020**, *9.* **[crossref], [Google Scholar], [Publisher]**
[8] M. Shirvani, F. Soufi, A. Nouralishahi, K. Vakili, A. Salimi, B. Lucke-Wold, F. Mousavi, S. Mohammadzadehsaliani, S. Khanzadeh, *BioMed Research International,* **2022**, *15.* **[crossref], [Google Scholar], [Publisher]**
[9] H. Bastami, M. Panahi, *International Journal of Advanced Studies in Humanities and Social Science*, **2020**, *9*, 97-111. **[Crossref], [Google Scholar], [Publisher]**
[10] H. Kheradjoo, A. Nouralishahi, M.S. Hoseinzade Firozabdi, S. Mohammadzadehsaliani, *Nanomedicine Research Journal*, **2022**, *7,* 264-269. **[Crossref], [Google Scholar], [Publisher]**
[11] S. Mohammadzadehsaliani, N. Kargar Dehnavi, A. Nouralishahi, H. Kheradjoo, *Academic Journal of Health Science*, **2023**, *38,* 121-124 **[Google Scholar], [Publisher]**
[12] H. Jabbari, *The Open Medicinal Chemistry Journal*, **2022**, *15.* **[Crossref], [Google Scholar], [Publisher]**
[13] A. Forutan Mirhosseini, H. Jabbari, A. Nouralishahi, Z. Bahman, S. Yahyazadeh Jasour, A. and Nekouei, Nanomedicine *Research Journal*, **2022,** *7,* 254-263. **[Crossref], [Google Scholar], [Publisher]**
[14] M. Saliminasab, H. jabbari, H. Farahmand, M. Asadi, M. Soleimani, A. Fathi, *Nanomedicine Research Journal*, 2022, *7,* 391-396. **[Crossref], [Google Scholar], [Publisher]**
[15] A. Aghababai beni, H. Jabbari, *Results in Engineering*, **2022**, *15*, 100467. **[Crossref], [Publisher]**
[16] S. Abbasi, A. İlhan, H. Jabbari, P. Javidzade, M. Safari, F. Abolhasani Zadeh, *Nanomedicine Research Journal*, **2022**, *7,* 156-164. **[Crossref], [Google Scholar], [Publisher]**
[17] J.R. Liu, M.M. Kadhim, M.J. Ansari, A.G. Ebadi, *Computational and Theoretical Chemistry,* **2022,** *1212,* 113729. **[Crossref], [Google Scholar], [Publisher]**
[18] H. Jabbari, *Journal of Molecular Biology Research,* **2020,** *10, 166-166.* **[Crossref], [Google Scholar], [Publisher]**
[19] H. Jabbari, R. Shendabadizad, , *Journal of Advanced Pharmacy Education & Research*, **2020**. **[Google Scholar], [Publisher]**
[20] H. Jabbari, *Arch. Pharm. Pract*, **2020**, *1,* 71-75. **[Google Scholar], [Publisher]**
[21] H. Ghian, N. Noroozi, H. Jabbari, *Communications in Catalysis*, **2018,** *1,* 20-31. **[Crossref], [Google Scholar], [Publisher]**
[22] H. Jabbari, N.N. Pesyan, *Asian Journal of Green Chemistry,* **2017**, *1,* 41-45. **[Google Scholar]**
[23] H. Jabbari, N. Noroozi Pesyan, *Asian Journal of Green Chemistry*, **2017**, *1,* 16-23. **[Google Scholar]**
[24] H. Jabbari, *Communications in Catalysis*, **2018**, *1,* 11-19. **[Crossref], [Google Scholar], [Publisher]**
[25] S. Mohammadi-Aghdam, H. Jabbari, O. Pouralimardan, F. Divsar, I. Amini, S. Sajjadifar, *Quarterly Journal of Iranian Chemical Communication,* **2019**, *7,* 15-28. **[Crossref], [Google Scholar], [Publisher]**
[26] H. Jabbari, J. Khalafy, P. Najafi Moghadam, *Quarterly Journal of Iranian Chemical Communication*, **2015**, *9,* 180-282. **[Google Scholar], [Publisher]**
[27] S. Khosravaniardakani, *International Journal of Medical Investigation*, **2022,** *11*. **[Google Scholar], [Publisher]**
[28] S. Khosravaniardakani, D.O. Bokov, T. Mahmudiono, S.S. Hashemi, N. Nikrad, *Frontiers in nutrition*, **2022**, *9*. **[Crossref], [Google Scholar], [Publisher]**
[29] S.A. Ahadiat, M. Shirazinia, S. Shirazinia, S. Garousi, M. Mottahedi, A.B. Jalali, A.B. Dahooiyeh, E. Pourmohammadi-Shahrbabaki, H. Baghsheikhi, A. Afzalian, S. Varshochi, *Kindle,* **2022**, *2,* 1-191. **[Google Scholar], [Publisher]**
[30] S. Khosravaniardakani, *International Journal of New Chemistry*, **2022**, *9,* 102-107. **[Google Scholar], [Publisher]**
[31] S. Khosravaniardakani, M.H. Anbardar, S. Khodamoradi, S. Peyravi, *International Journal of Medical Investigation*, **2022**, *11*. **[Google Scholar], [Publisher]**

[32] A. Johnson, A. Brous, A. samimi, *Progress in chemical and Biological Research*, **2022**, *5,* 218-228. **[Crossref], [Publisher]**

[33] F. Rebout, A. Samimi, *Progress in Chemical and Biochemical Research,* **2022**. *5,* 196-217. **[Google Scholar]**

[34] A. Johnson, A. Brous, A. Samimi*, Advanced Journal of Chemistry-Section B: Natural Products and Medical Chemistry,* **2022***, 4, 124*. **[Google Scholar]**

[35] A. Samimi, *Bulletin of Pure & Applied Sciences-Chemistry*, **2013**, *32,* 51-59. **[Google Scholar], [Publisher]**

[36] S.A. Mahkooyeh, S. Eskandari, E. Delavar, M. Milanifard, F.E. Mehni, *Eurasian Chemical Communications*, **2022**, 338-346. **[crossref], [Google Scholar], [Publisher]**

[37] A.A. Esmaeilzadeh, M. Kashian, H.M. Salman, M.F. Alsaffar, M. Musa Jaber, S. Soltani, D. Amiri Manjili, A. Ilhan, A. Bahrami, J.W. Kastelic, *Biology*, **2022**, *11*, 1851. **[crossref], [Google Scholar], [Publisher]**

[38] A.A. Esmaeilzadeh, S. Rasoolzadegan, A.R. Arabi, D. Soofi, S.S Rajaei Ramsheh, W. Saad Ahmed, R. Moaref Pour, *Nanomedicine Research* Journal, **2022,** *7,* 370-377. **[crossref], [Google Scholar], [Publisher]**

[39] A.A. Esmaeilzadeh, M.G. Pisheh Sanani, M. Amirahmadi, A.M. Tajik, M. Farsizaban**,** *Archives of Pharmacy Practice*, **2020**, *11,* 35. **[Google Scholar], [Publisher]**

[40] D. Birman, *Eurasian Journal of Chemical, Medicinal and Petroleum Research,* **2023**, *2,* 24-36. **[crossref], [Google Scholar], [Publisher]**

[41] E. Ghaibi, M.R. Soltani Manesh, H. Jafari Dezfouli, F. Zarif, Z. Jafari, Z. Gilani, *Eurasian Journal of Chemical, Medicinal and Petroleum Research,* **2022**, *1,* 33-39. **[crossref], [Google Scholar], [Publisher]**

[42] E. Ghaibi, M.R. Soltani Manesh, M. Bushra, Z. Gilani, K. Salimi Nabi, F. Zarif, *Eurasian Journal of Chemical, Medicinal and Petroleum Research*, **2022**, *1,* 49-57. **[crossref], [Google Scholar], [Publisher]**

[43] F. Mirakhori, M. Moafi, M. Milanifard, A. Asadi rizi, H. Tahernia, *Journal of Pharmaceutical Negative Results,* **2022**, *13*, 1889-1907. **[Crossref], [Google Scholar], [Publisher]**