

Original Article

Evaluation of Biological Markers in Children's Cerebrospinal Fluid with Bacterial and Viral Meningitis

Mohammad Panji¹ Ph.D., Vahideh Behmard² Ph.D., Mehri Fallah Raoufi³ Ph.D., Elnaz Sadat Mousavi Sadr Jadidi⁴ Ph.D., Elham Rostami⁵ Ph.D., Rahil Norbakhsh⁶ Ph.D., Vajiheh Najafi⁷ Ph.D., Tohid Moradi Gardeshi⁸ Ph.D., Fahimeh Safizadeh⁹ Ph.D., Haleh Barmaki^{10*} Ph.D.

¹ Department of Molecular Medicine, Faculty of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran

² Student Research Committee, Department of Midwifery, Faculty of Medicine, Gonabad University of Medical Sciences, Gonabad, Iran

³ Department of Clinical Science, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

⁴ Department of Basic Sciences, Payame Noor University, Tehran, Iran

⁵ Department of Biology, Faculty of Science, Shahid Chamran University of Ahvaz, Ahvaz, Iran

⁶ Department of Virology, Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

⁷ Research Center for Biochemistry and Nutrition in Metabolic Diseases, Kashan University of Medical Sciences, Kashan, Iran

⁸ Department of Veterinary Sciences, Garmsar Branch, Islamic Azad University, Garmsar, Iran

⁹ Iranian Social Security Organization, Kerman, Iran

¹⁰ Department of Laboratory Medicine, Faculty of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran

ABSTRACT

Article history

Received: 2 Jul 2023

Accepted: 14 Aug 2023

Available online: 30 Sep 2023

Keywords

Bacterial meningitis

Biological marker

Cerebrospinal fluid

Viral meningitis

Background and Aims: Cerebrospinal fluid (CSF) and blood biomarkers are widely evaluated as diagnostic tools to distinguish between bacterial meningitis and viral meningitis in emergency laboratory tests. So, this aimed to examine the levels of diagnostic parameters in blood and CSF to discriminate between bacterial and viral meningitis in young children referred to Khoy Hospital.

Materials and Methods: A total of 101 young children with meningitis were enrolled in this prospective study. The diagnosis of bacterial and viral meningitis was based on clinical features and laboratory findings.

Results: Of the 101 patients with meningitis, 18 (17.82%) were bacterial, and 83 (82.17%) were viral meningitis. The levels of CSF glucose and CSF/blood glucose ratio were significantly lower in the bacterial meningitis group than in the viral meningitis group ($p < 0.01$). In contrast, white blood cells count, CSF protein, interleukin-6, C-reactive protein, and ferritin levels were significantly higher in patients with bacterial meningitis compared to viral meningitis ($p < 0.01$). However, there was no difference between viral and bacterial meningitis groups concerning mean serum glucose.

Conclusion: This study suggests that decreased CSF glucose and CSF/blood glucose ratio and increased white blood cells count, CSF protein, ferritin, interleukin-6, and C-reactive protein combined with clinical symptoms can help better diagnosis of bacterial meningitis, especially in comparison with viral meningitis.

Introduction

Meningitis disorder is one of the severe infections of the central nervous system (CNS) in both newborns and infants [1]. Acute bacterial meningitis is a progressive bacterial infection of the meninges and subarachnoid space, which occurs in about 10% of meningitis cases [2, 3]. This acute meningeal infection develops in individuals of all ages and leads to morbidity and mortality [2]. In patients with bacterial meningitis who receive appropriate medical care and antibiotic medication, the risk of death is less than 15% [4, 5]. In children, classic manifestations of meningitis include severe headache, fever, photophobia, nausea, vomiting, back pain, irritability, and lethargy, which may develop over hours or up to a day [2, 6]. About 20% of patients will experience a seizure before hospitalization [2].

In contrast, studies report that 90% of all meningitis cases show a non-bacterial etiology [7, 8]. Viral meningitis or aseptic meningitis occurs due to a viral infection, and patients with viral meningitis do not require any antibacterial treatment and rarely require hospitalization [9, 10]. Diagnosis of meningitis type is essential to reduce the unnecessary use of antivirals or antibiotics and hospitalization. When diagnosis and medical treatment are delayed, severe neurological sequelae occur after bacterial meningitis in children [11, 12]. So, an excellent diagnostic application must attain a sensitivity near 100% [3, 13]. Currently, some clinical criteria and the classic parameters like C-reactive protein (CRP) level,

glucose level, neutrophil count, and white blood cell (WBC) count in blood and glucose level, protein level, and WBC count in cerebrospinal fluid (CSF) offer good sensitivity and specificity for discriminating between bacterial and viral meningitis. However, these parameters do not offer adequate specificity [14-16]. Thus, the exploration of other potential parameters remains a significant challenge. Multiple studies in bacterial meningitis suggest that inflammation is more common in bacterial than in viral meningitis. Ferritin is an acute-phase protein that cannot cross the blood-brain barrier, and the CSF level of ferritin increases during the inflammatory process and bacterial meningitis [1, 17]. Inflammatory cytokines such as tumor necrosis factor- α and interleukin (IL)-6 have been reported to augment the concentration of ferritin in both blood and CSF [1, 18, 19]. So far, very limited studies have been conducted in the field of effective biomarkers discriminating between bacterial and viral meningitis in Iranian children. According to the above, the objective of the current manuscript was to compare clinical features and the levels of diagnostic biomarkers in blood and CSF to distinguish between bacterial and viral infection in children and infants referred to as Khoy Hospital.

Materials and Methods

A total of 18 children with bacterial meningitis and 83 children with viral meningitis referred

to Qamar Bani Hashem Hospital in Khoy (between 2018 and 2020) were enrolled in this prospective study. Before the study, informed consent was obtained from the parents of all participating children to participate in this study. Meningitis was diagnosed based on physical examination, bacterial culture and gram staining, patient history, and CSF laboratory analysis. A flow chart for diagnosing bacterial and viral meningitis in children is presented in Figure 1. Overall, bacterial meningitis is defined according to the following findings: leukocyte count $> 1500/\mu\text{l}$, increased protein $> 2 \text{ g/l}$, decreased CSF glucose, positive bacterial culture, and/or gram staining. However, meningitis is defined as viral if any bacterial meningitis criteria is absent. The viral meningitis criteria were CSF neutrophilic pleocytosis, normal CSF protein and glucose, and negative gram staining and/or bacterial culture. According to standard criteria, patients categorized neither as bacterial nor viral meningitis were excluded from this study. Routine methods investigated the levels of glucose, peripheral white blood cell (WBC) count, and qualitative CRP in blood and glucose and protein levels in CSF samples. CSF WBC count, CSF lymphocyte, and neutrophil count were checked with a cell counter (Sysmex- K-800, Japan). Glucose levels were determined by Autoanalyzer (Hitachi 917, Japan) with the use of commercially available kits (Pars Azmoon, Tehran). Total protein levels in CSF were determined by Q-Portable Turbidimeter HACH:2100Q01. The enzyme immunoassay method measured CSF ferritin, and the hs-

CRP value was detected using immunoturbidimetry by the Hitachi 911 device. CSF IL-6 was measured through the enzyme-linked immunosorbent assay technique (R&D Systems, Minneapolis, MN).

The present study was approved by the ethical committee of Khoy University of Medical Sciences, Khoy, Iran and the investigation results from a research project of the Qamar Bani Hashem Hospital in Khoy through the following approved code: A-1397/03.

Statistical analysis

Data were analyzed using the SPSS software (version 16). The results are representative of three independent experiments and reported as mean \pm standard deviation (SD). Due to the abnormal distribution of data and the small number of samples, non-parametric tests were used. Mann-Whitney test was used to compare quantitative variables between the two groups, and Fisher's exact test was used to compare qualitative variables between the two groups. $p < 0.05$, was considered as a significant level.

Results

Of the 101 patients with meningitis, 18 (17.82%) were bacterial, and 83 (82.17%) had viral meningitis. In the group of patients with bacterial meningitis, the mean age was 13.3 ± 12.4 (range, 1 to 30 months), and in the group with viral meningitis, the mean age was 14.09 ± 12.25 (range, 1.5 to 32 months). The clinical feature (%) and laboratory diagnostic parameters in both bacterial and viral meningitis groups are summarized in Tables 1 to 3. As shown in Table 1, the most common clinical features of meningitis in the two study

groups were vomiting, fever, headache, diarrhea, convulsion, and CRP. Fever was observed in 100% of both bacterial and viral meningitis cases. The mean \pm SD for WBC, CSF lymphocyte and neutrophil counts, CSF protein levels, CSF glucose levels, serum glucose, and CSF/blood glucose ratio in both groups are shown in Table 2. The differences in the mean \pm SD were statistically significant for WBC, CSF glucose, CSF protein, and CSF/blood glucose ratio between two viral meningitis and bacterial meningitis groups. The mean of CSF glucose and CSF/blood glucose ratio was significantly lower in the bacterial meningitis group than in the viral meningitis group. In contrast, WBC counts and CSF protein was significantly higher in patients with bacterial meningitis than in viral meningitis ($p < 0.01$). There was no difference between viral meningitis and bacterial

meningitis groups concerning mean neutrophil count, lymphocyte counts, and serum glucose. Bacterial culture showed *Streptococcus pneumonia* in one case, Group B *hemolytic streptococci* in one case, *Neisseria meningitides* in five cases, *Haemophilus influenzae* in six cases, *Escherichia coli* in four cases, and *Pseudomonas auroginosa* in one case (Table 3). The mean CSF IL-6 in bacterial meningitis was 180.74 pg/ml and in viral, it was 39.08 pg/ml ($p=0.000$). The mean CRP of CSF in bacteria was 55.22 ± 3.11 mg/L, and in viral 7.5 ± 1.18 mg/L. CSF IL-6 and CRP concentrations were significantly higher in bacterial than in viral meningitis. Moreover, the mean ferritin CSF in children with bacterial meningitis (106.39 ± 86.96 ng/dl) is higher than in patients with viral meningitis (10.17 ± 14.09 ng/dl) (Table 4).

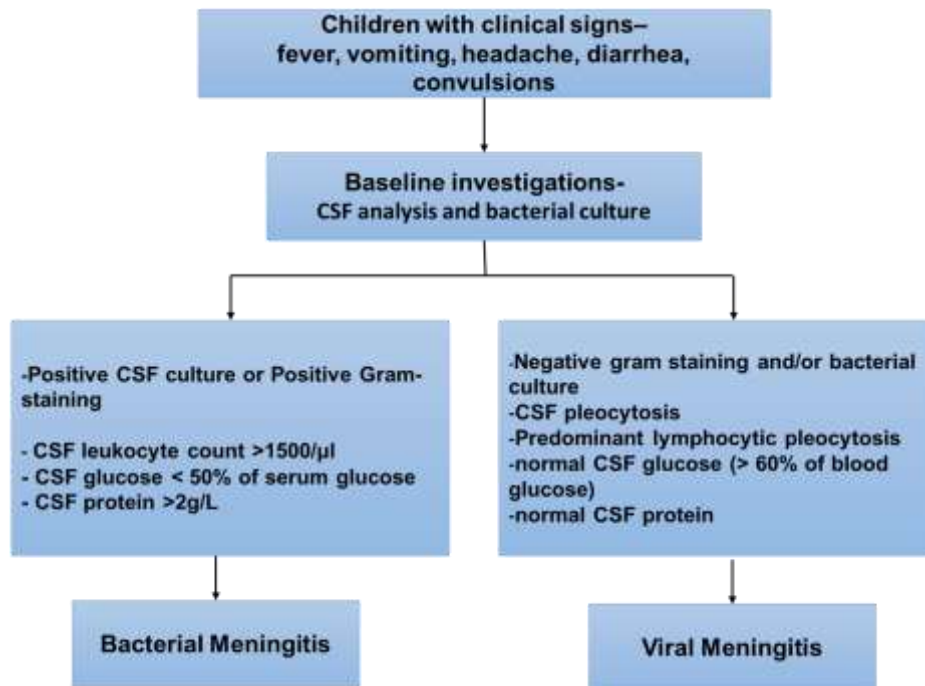


Fig. 1. Flow chart for diagnosing meningitis in children
CSF= Cerebrospinal fluid

Table 1. The comparison of C-reactive protein, vomiting, headache, fever, diarrhea, and convulsions between bacterial meningitis and viral meningitis groups

Clinical feature	Bacterial meningitis (18)		Viral meningitis (83)	
	Yes	No	Yes	No
C-reactive protein	9 (50%)	9 (50%)	34 (40.9%)	49 (59.1%)
Vomiting	11 (61.1%)	7 (38.9%)	37 (44.5%)	46 (55.5%)
Headache	1 (5.6%)	17 (94.4)	11 (13.3%)	72 (86.7%)
Fever	18 (100%)	0 (0)	83 (100%)	0 (0)
Diarrhea	3 (16.7%)	15 (83.3%)	9 (10.8%)	74 (89.2%)
Convulsions	6 (33.3%)	12 (66.7%)	25 (30.1%)	58 (69.9%)

Data are presented as N (%)

Table 2. The comparison of CSF WBC, CSF neutrophil counts, CSF lymphocyte counts, CSF protein levels, CSF glucose levels, serum glucose level, and CSF/blood glucose ratio between bacterial meningitis and viral meningitis groups

Parameters	Diagnosis	Bacterial meningitis (18)	Viral meningitis (83)	P
		Mean±SD	Mean±SD	
CSF white blood cells (/µL)		22149±3675.28	4549.2±986.21	0.001
CSF neutrophil		61.38±26.66	54.46±21.42	0.31
CSF lymphocyte		38.62±26.66	45.54±21.42	0.23
CSF Glucose (mg/dL)		26.83±10.93	61.12±13.81	0.000
CSF Protein(gr/L)		1510.6±236.17	63.51±82.36	0.007
Serum Glucose (mg/dl)		111.28±54.13	102.89±34.45	0.4
Cerebrospinal fluid/blood glucose ratio		0.36±0.24	0.65±0.29	0.001

p≤0.05 was considered as a significant level. CSF= Cerebrospinal fluid

Table 3. Results of cerebrospinal fluid culture of patients.

Parameters	Diagnosis	Bacterial meningitis (18)	Viral meningitis (83)
Culture			
<i>Streptococcus pneumonia</i>		1	
<i>Group B hemolytic streptococci</i>		1	
<i>Hemophilus influenza</i>		6	Not applicable
<i>Neisseria meningitides</i>		5	
<i>Escherichia coli</i>		4	
<i>Pseudomonas aeruginosa</i>		1	

Table 4. The comparison of high sensitive C - reactive protein, interleukin-6, and ferritin concentrations in cerebrospinal fluid of patients with bacterial meningitis and viral meningitis.

Parameters	Diagnosis	Bacterial meningitis (18)	Viral meningitis (83)	P
		Mean±SD	Mean±SD	
C-reactive protein (mg/L)		55.22 ± 3.11	7.5 ± 1.18	0.001
Interlukin-6 (pg/ml)		180.74 ± 121.05	39.08 ± 29.6	0.001
Ferritin (ng/dl)		106.39±86.96	10.17±14.09	0.001

p ≤ 0.05 was considered as a significant level.

Discussion

The current study reports that the most common clinical features for meningitis in the two study groups were vomiting, fever, headache, diarrhea, convulsion, and CRP. We also showed that the mean glucose level in CSF and CSF/blood glucose ratio was significantly lower in the bacterial meningitis group than in the viral meningitis group. Simultaneously, CSF protein, WBC counts, CRP, and ferritin were significantly higher in patients with bacterial meningitis than in viral meningitis.

In the clinic, the differential distinction between bacterial and viral meningitis is often very complicated. In recent decades, pneumococcus has been considered the primary cause of bacterial meningitis in children. According to the statistical analyses, the incidence of *pneumococcus meningitis* in young children was 36% [20-22]. Therefore, various clinical features can help the early diagnosis of acute bacterial meningitis by a physician [23]. It is mentionable that the predominant clinical features of bacterial meningitis in young children and neonates were characterized by fever, poor feeding, irritability, lethargy, vomiting, and seizures [24, 25]. In the present study, we showed that the most common clinical features for bacterial meningitis were vomiting, fever, headache, diarrhea, convulsion, and CRP, with 61.1%, 100%, 5.6%, 11.1%, 33.3%, and 50% frequency, respectively. Fever was the predominant clinical feature (100%) in bacterial meningitis and viral meningitis. Also,

vomiting, diarrhea, convulsion, and increased CRP occurred more frequently in children with bacterial meningitis than in viral meningitis cases.

There is a slight decrease in CSF glucose in viral meningitis and a mild increase in WBC count, mostly lymphocytes [9, 26]. On the other hand, it is considered that laboratory findings in bacterial meningitis samples were characterized by decreased CSF glucose and glucose ratio in CSF to glucose in serum, increased CSF leukocyte counts, and increased CSF protein [24, 27, 28]. Tamune et al. suggested that decreased glucose levels in CSF and CSF/blood glucose ratio can predict the presence of bacterial meningitis [29]. In 2021, Chamkhaleh et al. showed that decreased glucose level in CSF was a characteristic of bacterial meningitis. However, increased CSF protein was reported as a significant mortality risk factor in Iranian children with meningitis [30]. In a current study, Ahmed et al. found that the CSF/blood glucose ratio was significantly lower among patients with bacterial meningitis. They also reported that patients with bacterial meningitis had notably higher CSF protein in comparison to those with viral meningitis [31]. Tan and his colleagues reported that high protein levels in CSF are related to poor outcomes in neonates with bacterial meningitis [32]. Many studies have shown that high protein levels in CSF are associated with severe inflammatory reactions and later poor outcomes in patients with bacterial meningitis [33-35]. In contrast, CSF

protein and glucose levels are not affected by a systemic inflammatory response syndrome if there is no meningitis. This finding is consistent with the results of our study. Here, we showed that the mean of CSF glucose and CSF/blood glucose ratio was significantly lower in the bacterial meningitis group than in the viral group. At the same time, CSF protein and WBC counts were significantly higher in patients with bacterial meningitis compared to viral meningitis. There was no difference between viral meningitis and bacterial meningitis groups concerning mean serum glucose.

Previous studies suggest that CSF-specific inflammatory markers and CSF ferritin are essential biomarkers for early meningitis diagnosis [1, 36, 37]. We measured two inflammatory parameters, IL-6 and CRP, in the CSF of patients, and finally, the concentrations of these two markers in two groups of patients were compared. So, IL-6 and CRP concentrations in CSF were remarkably elevated in patients with bacterial meningitis compared to patients with viral meningitis. IL-6 is a multifunctional cytokine that acts as a mediator in meningeal inflammation and acts in hypothalamic regions of the brain to prompt fever. Xu et al. evaluated CSF concentrations of cytokines IL-6, IL-10, tumor necrosis factor- α , and interferon- γ in children with bacterial and viral meningitis. According to their findings, the levels of these cytokines showed no difference between the two groups [36]. The difference between the results of Xu's study and our study may be due to the number of patients included

in the studies. Abyaz et al. found that the measurement of the serum levels of CRP could not be a useful tool for differentiating bacterial meningitis from non-meningitis patients with similar clinical symptoms [38]. Unlike serum CRP, we showed the capability of CSF CRP to distinguish between bacterial and viral meningitis in Iranian children.

Measurement of CSF ferritin has also been considered a useful screening test in the early diagnosis of bacterial meningitis and has some diagnostic value in distinguishing bacterial from viral meningitis [1, 39]. Ferritin is the principal intracellular storage protein of iron and cannot pass the healthy blood-brain barrier, and therefore blood ferritin levels do not influence ferritin concentration in CSF [40]. According to findings from the present study, CSF ferritin concentration was significantly higher in bacterial meningitis than in viral meningitis.

Conclusion

This research demonstrates that CSF markers, combined with other clinical signs and symptoms, can help diagnose and differentiate bacterial and viral meningitis. In the future, more extensive studies with more samples are recommended to achieve more reliable results.

Conflict of Interest

The authors declare that there is no conflict of interest associated with this work.

Acknowledgments

We would like to thank the Faculty of Medical Sciences of Qamar Bani Hashem Hospital for their support.

References

- [1]. Jebamalar AA, Prabhat Balakrishnapillai AK, Parmeswaran N, Dhiman P, Rajendiran S. Cerebrospinal fluid ferritin and albumin index: potential candidates for scoring system to differentiate between bacterial and viral meningitis in children. *Biomarkers* 2016; 21(5): 424-28.
- [2]. Davis LE. Acute bacterial meningitis. *Contin Lifelong Learn Neurol*. 2018;24(5): 1264-283.
- [3]. Boskabadi H, Heidari E, Zakerihamidi M. Etiology, clinical findings and laboratory parameters in neonates with acute bacterial meningitis. *Iran J Microbiol*. 2020; 12(2): 89.
- [4]. Kietzman C, Tuomanen E. Acute bacterial meningitis: challenges to better antibiotic therapy. *ACS Infect Dis*. 2019; 5(12): 1987-995.
- [5]. Griffiths MJ, McGill F, Solomon T. Management of acute meningitis. *Clin Med*. 2018; 18(2): 164.
- [6]. Viallon A, Botelho-Nevers E, Zeni F. Clinical decision rules for acute bacterial meningitis: current insights. *Open Access Emerg Med*. 2016; 8(1): 7-16.
- [7]. Chusri S, Hortiwakul T, Sathaporn N, Charernmak B, Phengmak M, Jitpi boon W, et al. Diagnostic scoring system for tuberculous meningitis among adult patients with non-suppurative and non-bacterial meningitis. *J Infect Chemother*. 2018; 24(8): 648-53.
- [8]. Panji M, Behmard V, Zare Z, Malekpour M, Nejadbiglari H, Yavari S, et al. Synergistic effects of green tea extract and paclitaxel in the induction of mitochondrial apoptosis in ovarian cancer cell lines. *Gene* 2021; 787: 145638.
- [9]. McGill F, Griffiths MJ, Solomon T. Viral meningitis: current issues in diagnosis and treatment. *Curr Opin Infect Dis*. 2017; 30(2): 248–56.
- [10]. Alaei Sheini F, Tabnak M, Hasanzadeh Bezvan M, Mahdiannasser M, Musavi H, Choobineh H, et al. A systematic review of the evidence on the effects of cytomegalovirus on abortion. *Int J Med Lab*. 2018; 5(3): 173–81.
- [11]. Svendsen MB, Ring Kofoed I, Nielsen H, Schønheyder HC, Bodielsen J. Neurological sequelae remain frequent after bacterial meningitis in children. *Acta Paediatr*. 2020; 109(2): 361–67.
- [12]. Shahidi M, Abazari O, Dayati P, Reza JZ, Modarressi MH, Tofighi D, et al. Using chitosan-stabilized, hyaluronic acid-modified selenium nanoparticles to deliver CD44-targeted PLK1 siRNAs for treating bladder cancer. *Nanomedicine* 2023; 18(3): 259-77.
- [13]. Musavi H, Abazari O, Safaee MS, Variji A, Koohshekan B, Kalaki-Jouybari F, et al. Mechanisms of COVID-19 entry into the cell: potential therapeutic approaches based on virus entry inhibition in COVID-19 patients with underlying diseases therapeutic approaches based on SARS-COV-2 entry inhibition. *Iran J Allergy, Asthma Immunol*. 2021; 20(1): 11-19.
- [14]. Honda T, Uehara T, Matsumoto G, Arai S, Sugano M. Neutrophil left shift and white blood cell count as markers of bacterial infection. *Clin Chim Acta*. 2016; 457: 46–53.
- [15]. Njunge JM, Oyaro IN, Kibinge NK, Rono MK, Kariuki SM, Newton CR, et al. Cerebrospinal fluid markers to distinguish bacterial meningitis from cerebral malaria in children. *Wellcome open Res*. 2017; 2(1): 47.
- [16]. Shahidi M, Moradi A, Dayati P. Zingerone attenuates zearalenone-induced steroidogenesis impairment and apoptosis in TM3 Leydig cell line. *Toxicon* 2022; 211(1): 50–60.
- [17]. Shokrollahi MR, Shabanzadeh K, Noorbakhsh S, Tabatabaei A, Movahedi Z, Shamshiri AR. Diagnostic value of CRP, procalcitonin, and ferritin levels in cerebrospinal fluid of children with meningitis. *Cent Nerv Syst Agents Med Chem*. 2018; 18(1): 58-62.
- [18]. Abbasi M, Namjoo AR, Khamesipour F. Ethanol effects on histobiochemical parameters of suckling pups borned from alcoholic rat mothers. *Comp Clin Path*. 2016; 25(4): 833-39.
- [19]. Karimi R, Bakhshi A, Dayati P, Abazari O, Shahidi M, Savaee M, et al. Silymarin reduces retinal microvascular damage in streptozotocin-induced diabetic rats. *Sci Rep*. 2022; 12(1): 1-14.
- [20]. Buchholz G, Koedel U, Pfister HW, Kastenbauer S, Klein M. Dramatic reduction of mortality in pneumococcal meningitis. *Crit Care*. 2016; 20(1): 1-9.
- [21]. Asadi A, Nezhad DY, Javazm AR, Khanicheragh P, Mashouri L, Shakeri F, et al. In vitro effects of curcumin on transforming growth factor- β -mediated non-smad signaling pathway, oxidative stress, and pro-inflammatory cytokines production with human vascular smooth muscle cells. *Iran J Allergy, Asthma Immunol*. 2020; 2(1): 84-93.
- [22]. Musavi H, Fattah A, Abbasi M. Differential expression of the KLK2 and KLK3 genes in peripheral blood and tissues samples of Iranian patients with prostate cancer. *Med Lab J*. 2019; 13(3): 25-30.
- [23]. van de Beek D, Cabellos C, Dzapova O, Esposito S, Klein M, Kloek AT, et al. ESCMID guideline: diagnosis and treatment of acute bacterial meningitis. *Clin Microbiol Infect*. 2016; 22(1): 37–62.
- [24]. Houri H, Pormohammad A, Riahi SM, Nasiri MJ, Fallah F, Dabiri H, et al. Acute bacterial meningitis in Iran: Systematic review and meta-analysis. *PLoS One*. 2017; 12(2): 169617.

- [25]. Musavi H, Abazari O, Barartabar Z, Kalaki-Jouybari F, Hemmati-Dinarvand M, Esmaeili P, Mahjoub S. The benefits of Vitamin D in the COVID-19 pandemic: biochemical and immunological mechanisms. *Arch Physiol Biochem.* 2023; 129(2): 354-62.
- [26]. Shahidi M, Abazari O, Dayati P, Bakhshi A, Rasti A, Haghirsadat F, et al. Aptamer-functionalized chitosan-coated gold nanoparticle complex as a suitable targeted drug carrier for improved breast cancer treatment. *Nanotechnol Rev.* 2022; 11(1): 2875-890.
- [27]. Zare Z, Dizaj TN, Lohrasbi A, Sheikhalishahi ZS, Asadi A, Zakeri M, et al. Silibinin inhibits TGF- β -induced MMP-2 and MMP-9 through Smad Signaling pathway in colorectal cancer HT-29 cells. *Basic Clin Cancer Res.* 2020; 12(2): 81-90.
- [28]. Fattah A, Morovati A, Niknam Z, Mashouri L, Asadi A, Rizi ST, et al. The synergistic combination of cisplatin and piperine induces apoptosis in MCF-7 cell line. *Iran J Public Health.* 2021; 50(5): 1037-1047.
- [29]. Tamune H, Takeya H, Suzuki W, Tagashira Y, Kuki T, Honda H, et al. Cerebrospinal fluid/blood glucose ratio as an indicator for bacterial meningitis. *Am J Emerg Med.* 2014; 32(3): 263-66.
- [30]. Chamkhaleh MA, Noorbakhsh S, Vafae-Shahi M, Riahi A, Hajinasab N, Gandomi-Mohammadabadi A, et al. The epidemiology and outcomes of meningitis among iranian children in a period of 10 years. *Open Neurol J.* 2021; 15(1): 37-42.
- [31]. Ahmed MA, Askar GA, Farghaly HS, Ahmed AO, Kamal DT, Ahmed SS, et al. Accuracy of cerebrospinal fluid c-reactive protein and multiplex polymerase chain reaction and serum procalcitonin in diagnosis of bacterial and viral meningitis in children. *Acta Neurol Taiwan* 2022; 31(1): 61-71.
- [32]. Tan J, Kan J, Qiu G, Zhao D, Ren F, Luo Z, et al. Clinical prognosis in neonatal bacterial meningitis: the role of cerebrospinal fluid protein. *PLoS One* 2015; 10(10): 141620.
- [33]. Shahidi M, Abazari O, Dayati P, Haghirsadat BF, Oroojalian F, Tofighi D. Targeted delivery of 5-Fluorouracil, miR-532-3p and si-KRAS to colorectal tumor using Layer-by-Layer liposomes. *Front Bioeng Biotechnol.* 2022; 10: 1013541.
- [34]. Hsu MH, Hsu JF, Kuo HC, Lai MY, Chiang MC, Lin YJ, et al. Neurological complications in young infants with acute bacterial meningitis. *Front Neurol.* 2018; 10(9): 903.
- [35]. Mohammadi Tahroodi F, Abdyazdani N, Shakeri F, Rahmani M, Afrazian MS, Abbasi M, et al. The prevalence of hepatitis B, hepatitis C and AIDS in blood donors in Ilam Province: A retrospective study. *Iranian Journal of Virology* 2018; 12(1): 32-9.
- [36]. Xu J, Jiang J, Zhang Y, Li W. Cytokine characteristic of cerebrospinal fluid from children with enteroviral meningitis compared to bacterial meningitis. *J Clin Lab Anal.* 2020; 34(5): 23198.
- [37]. Abazari O, Shakibae A, Shahriary A, Arabzadeh E, Hofmeister M. Hepatoprotective effects of moderate-intensity interval training along with ginger juice in an old male rat model. *Pflügers Arch J Physiol.* 2023; 475(4): 437-52.
- [38]. Abyaz S, Jalali MT, Ekrami A, Kaydani G, Bashiri Y. Investigate and comparison of serum level markers lactate, hsCRP and CDT for differentiating bacterial meningitis from non-meningitis patients with similar clinical symptoms. *J Res Med Dent Sci.* 2018; 6(3): 393-99.
- [39]. Shahidi M, Abazari O, Bakhshi A, Zavarreza J, Modarresi M, Haghirsadat F, et al. Multicomponent siRNA/miRNA-loaded modified mesoporous silica nanoparticles targeted bladder cancer for a highly effective combination therapy. *Front Bioeng Biotechnol.* 2022; (8)10: 949704.
- [40]. Zhang N, Yu X, Xie J, Xu H. New insights into the role of ferritin in iron homeostasis and neurodegenerative diseases. *Mol Neurobiol.* 2021; 58(6): 2812-823.