

Altered serum fucose and its diagnostic relevance in newly diagnosed leukemia patients

Received: 14/5/2025

Accepted: 29/6/2025

Jihan Wais Jaleel^{1*}Bakhtiar Muhialdin Ahmed²

Abstract

Background and objective: Altered fucosylation patterns have been implicated in cancer progression and immune evasion. This study aimed to evaluate serum fucose and specific fucose-related parameters in newly diagnosed leukemia patients and assess their diagnostic potential.

Methods: A case-control study was conducted on 65 males (33 leukemia patients, 32 healthy controls), aged 20–75. Blood samples were collected at Nanakali Hospital (Erbil) and analyzed by spectrophotometry to determine levels of Total Fucose (TF), Protein-Bound Fucose (PBF), Lipid-Associated Fucose (LAF), Protein-Bound Hexose (PBH), and Free Fucose (FF). Statistical analysis was performed using GraphPad Prism with a significance level of $P^* < 0.001$. Diagnostic performance was assessed using ROC analysis.

Results: Leukemia patients had significantly elevated serum TF (median: 173.3 mg/dl), PBF (10.17 mg/dl), FF (161.1mg/dl), and LAF (mean: 3.347 ± 1.076), while PBH levels were decreased (18.97mg/dl). ROC analysis showed strong diagnostic performance for TF (AUC = 0.989, sensitivity = 96.97%, specificity = 90.63%) and FF (AUC = 0.986, sensitivity = 93.94%, specificity = 90.63%).

Conclusion: Fucose-related parameters, particularly TF and FF, are significantly altered in male leukemia patients and may serve as useful non-invasive diagnostic biomarkers.

Keywords: Glycoproteins, Glycolipids, Leukemia, Serum L-fucose, Serum protein-bound fucose, Serum protein-bound hexose.

Introduction

Leukemia is a malignancy that arises from carcinogenic alterations in leukocytes during hematopoiesis, resulting in dysfunctional immune cells

and impaired bone marrow function (1). The underlying biological mechanisms include gene mutations, chromosomal translocations, and disruptions in signaling pathways, varying by leukemia subtype (2).

¹ Department of Clinical Biochemistry, College of Health sciences, Hawler Medical University, Kurdistan Region, Iraq.

² Department of Basic Science, College of Dentistry, Hawler Medical University, Kurdistan Region, Iraq.

Correspondence: jihan.wais1@hs.hmu.edu.krd

Copyright (c) The Author(s) 2022. Open Access. This work is licensed under a [Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License](https://creativecommons.org/licenses/by-nc-sa/4.0/).

Leukemia is classified by cell lineage (lymphoid or myeloid) and disease progression (acute or chronic), leading to four major types: Acute Lymphoid Leukemia (ALL), Acute Myeloid Leukemia (AML), Chronic Lymphocytic Leukemia (CLL), and Chronic Myeloid Leukemia (CML) (3, 4). The disease is multifactorial, involving genetic predisposition and environmental exposures such as ionizing radiation, benzene, prior chemotherapy, and genetic syndromes like Down syndrome (5). Clinical manifestations include signs of bone marrow failure, such as anemia, neutropenia, and thrombocytopenia, as well as organ infiltration, bone pain, hepatosplenomegaly, lymphadenopathy, and occasionally neurological symptoms (6). Treatment is individualized, depending on the patient's age, general health, and leukemia subtype (7).

Glycobiology, once a minor field, has emerged as central to understanding immune function, development, and disease. Carbohydrates, particularly oligosaccharides, are now recognized as key players in cell signaling, immune responses, and pathogen interactions (8, 9). Monosaccharides such as fucose are enzymatically incorporated into oligosaccharide chains via glycosyltransferases, forming glycoconjugates when attached to proteins and lipids (10). Fucose's unique L-configuration and lack of a hydroxyl group at carbon-6 facilitate its distinct

glycosidic linkages in glycoproteins and glycolipids (11). Fucosylated structures, including Lewis antigens, mediate immune recognition and cell adhesion (12). Fucosylation plays essential roles in physiological processes, such as fertilization and brain development (13, 14), as well as in host-pathogen interactions during infection (15).

Altered fucosylation is increasingly recognized as a hallmark of cancer. It contributes to tumor growth, metastasis, immune evasion, and cell signaling dysregulation (16). In oncology, fucose has diagnostic and prognostic potential. For example, core fucosylated α -fetoprotein (AFP) enhances diagnostic specificity for hepatocellular carcinoma compared to total AFP levels. Furthermore, increased core fucosylation of proteins such as E-cadherin and integrins is associated with decreased cell-cell adhesion and enhanced metastatic potential, highlighting the biological and clinical importance of fucosylation in cancer progression (17).

Research examining particular fucose fractions in newly diagnosed leukaemia patients, such as total fucose, free fucose, protein-bound fucose, lipid-associated fucose, and protein-bound hexose, is conspicuously lacking. Haematological malignancies are under-represented in previous studies, which primarily focus on solid tumors. It is still unclear how these fucose-related markers are connected with leukaemia

in terms of diagnosis, clinical associations, and mechanism. This study was restricted to male participants to reduce variability associated with sex-specific variations in glycosylation patterns. Although the study's internal validity is strengthened by this design, more research involving both genders and larger populations is advised to increase the research's portability.

Methods

Study Design and Population

This case-control study included 65 adult male participants (33 newly diagnosed leukemia patients and 32 healthy controls), aged 20-75 years. The study was conducted between October 2024 and May 2025. Blood samples were collected at Nanakali Hospital (Erbil), and biochemical testing was performed at the Research Center of Hawler Medical University.

The participants were divided into two groups:

- Group 1: 33 newly diagnosed male leukemia patients (prior to chemotherapy, radiation, or transfusion).
- Group 2: 32 age-matched healthy male controls.

Exclusion Criteria: Individuals with chronic or acute inflammatory diseases, prior cancer treatments, recent blood transfusions, females, or children were excluded from the study.

Sample Collection and Handling

Venous blood (5mL) was collected in gel tubes and allowed to clot at room temperature for 10-15 minutes. Samples were centrifuged at 3000rpm for 12 minutes to separate the serum (18). The serum was aliquoted into Eppendorf tubes and stored at -20°C until analysis (19).

Biochemical Assays and Principles

Serum parameters were measured using a Genesys/10S UV-Visible spectrophotometer, with reagents prepared from analytical-grade chemicals. The following parameters were assessed:

- Total Fucose (TF) was measured utilizing the Dishes and Shettels method. The technique depends on the direct interaction between concentrated sulphuric acid (H₂SO₄) and the components of the sample. Then the reactants were combined with cysteine, and the resulting colorimetric products were measured at 396 nm and 430 nm (20, 21).
- Protein-Bound Fucose (PBF) was determined using the Dische and Shettels technique. Serum proteins were precipitated using ethanol. The precipitated protein was resuspended in NaOH to make it soluble once more. A chromatic product was generated when fucose (in solution) reacted with a color developer (cysteine hydrochloride)

in a strongly acidic medium. The color intensity was quantified at 396 nm and 430 nm (20, 22).

- Lipid-Associated Fucose (LAF) was estimated depending on Katopodis' method. The entire amount of serum lipid was extracted using a 2:1 mixture of methanol and v/v chloroform. Then, the Dische&Shettles method was used to quantify the amount of fucose after the protein is precipitated by a phosphotungstic acid solution (20, 23).
- Protein-Bound Hexose (PBH): was assessed based on the interaction with Orcinol. 95% ethanol was used to precipitate the protein-carbohydrate complex's hexose components, which were then measured using the Orcinol reaction. Next, the absorbance was quantified at 520 nm using a spectrophotometric apparatus (23).
- Free Fucose (FF): Calculated using the formula: $FF = \text{Total Fucose} - (\text{PBF} + \text{LAF})$

Pilot Study

A preliminary pilot study involving 5 leukemia patients and 5 controls was conducted to standardize experimental conditions and generate reference ranges for each parameter. The data helped optimize the spectrophotometric protocols used in the full study.

Sample Size and Design Justification

The case-control design was selected to compare biochemical markers between patients and controls at diagnosis. A power analysis was performed using the G*Power software to determine an adequate sample size for the primary objective.

Statistical Analysis

All statistical analyses were performed using GraphPad Prism (version 10.2.2). To determine the distribution of the data, we performed normality testing using the Shapiro–Wilk test for each variable. Variables that followed a normal distribution were analyzed using the unpaired t-test, while non-normally distributed variables were compared using the Mann–Whitney U test. Data were expressed as (mean \pm standard deviation (SD)) for normally distributed variables, and as (median) for non-normal data. The Receiver Operating Characteristic (ROC) curve was used to assess the diagnostic performance of fucose-related parameters. The optimal cutoff value was determined using the Youden Index ($J = \text{Sensitivity} + \text{Specificity} - 1$). A P-value of <0.001 was considered statistically significant.

Ethical Considerations

The study protocol was reviewed and approved by the Ethics Committee of Hawler Medical University. Verbal informed consent was obtained from all participants after a full explanation of the study objectives and procedures.

The ethics committee permitted verbal consent in this context due to the observational, non-interventional nature of the study. (Approval no.: Sc. E.C. 11J2)

Results

The median value (50%), along with the 25th and 75th percentiles of serum total fucose, protein-bound fucose, protein-bound hexose, and free fucose, are listed in Tables 1, 2, 3, and 5. In contrast, the mean \pm standard deviation of lipid-associated fucose is shown in Table 4. The results indicate a significant difference between the case and control groups.

The median total fucose level is significantly elevated ($P < 0.0001$) in leukemia patients relative to healthy controls. Additionally, free fucose ($P < 0.0001$) and protein-bound fucose

($P = 0.0002$) were also increased in the leukemia patients. Nonetheless, the protein-bound hexose is significantly reduced ($P < 0.0001$) in leukemia patients relative to the control group.

The mean level of lipid-associated fucose was considerably elevated in leukemia patients relative to the control group ($P < 0.001$).

The Receiver Operating Characteristic (ROC) curve was employed to evaluate the effectiveness of total fucose and free fucose as diagnostic biomarkers by plotting sensitivity against 1-specificity across various thresholds. The Area Under the Curve (AUC) was calculated to evaluate the performance of the test, where values nearing 1 indicate outstanding discrimination between conditions, whereas values close to 0.5 suggest insufficient discrimination.

Table 1. Represents the result of Serum Total fucose in the Leukemia and Control Groups

Serum Total Fucose*	In leukemia patients	In control group	P-Value
25% Percentile	125.9	38.08	
Median (50%)	173.3	44.54	< 0.0001
75% Percentile	202.1	61.17	

* Data are analyzed using a Mann-Whitney U Test.

Table 2. Represents the result of Serum Protein-Bound Fucose in Leukemia and Control Groups

Serum Protein-Bound Fucose*	In leukemia patients	In control group	P-Value
25% Percentile	9.93	7.029	
Median (50%)	10.17	9.021	0.0002
75% Percentile	10.52	10.14	

* Data are analyzed using a Mann-Whitney U Test.

Table 3. Represents the result of Serum Protein-Bound Hexose in Leukemia and Control Groups

Protein-Bound Hexose*	In leukemia patients	In control group	P-Value
25% Percentile	13.38	21.73	
Median (50%)	18.97	56.53	< 0.0001
75% Percentile	24.76	63.22	

* Data are analyzed using a Mann-Whitney U Test.

Table 4. represents the result of Serum Lipid-Associated Fucose in Leukemia and Control Groups

Serum Lipid-Associated Fucose**	In leukemia patients	In control group	P-Value
Mean	3.347	1.366	<0.0001
Std. Deviation	±1.076	±0.6315	

** Data are analyzed using an independent T-test.

Table 5. represents the result of Serum Free Fucose in the Leukemia and Control Groups

Serum Free Fucose*	In leukemia patients	In control group	P-Value
25% Percentile	127.9	46.54	
Median (50%)	161.1	71.94	< 0.0001
75% Percentile	175.1	86.8	

* Data are analyzed using a Mann-Whitney U Test.

Table 6. illustrates the outcomes of the ROC curves

Biomarker	AUC	Cutoff-Value (mg/dL)	Sensitivity (%)	Specificity (%)	95% CI	P-value
Total Fucose	0.9896	> 98.04	96.97	90.63	84.68% to 99.84%	<0.000001
Free Fucose	0.9867	> 87.87	93.94	90.63	72.67% to 95.18%	<0.000001

Figure 1 presents the ROC curve for a. TF and b. FF, demonstrating that both TF and FF exhibit notable sensitivity and specificity. The analysis of TF revealed an AUC of 0.9896, with a cutoff value established at ($> 98.04\text{mg/dL}$), resulting in a sensitivity of 96.97% and a specificity of 90.63%. The analysis of free fucose resulted in an AUC of 0.9867, establishing a cutoff value of ($> 87.87\text{mg/dL}$), with a sensitivity of 93.9% and a specificity of 90.63%. Table 6 provides a concise summary of this information.

Discussion

This study revealed significant alterations in serum fucose and its related parameters in newly diagnosed male leukemia patients compared to healthy controls. Specifically, levels of total fucose (TF), protein-bound fucose (PBF), lipid-associated fucose (LAF),

and free fucose (FF) were elevated in the patient group. In contrast, protein-bound hexose (PBH) was reduced.

Fucosylated glycans, comprising glycoproteins and glycolipids, are important structural and functional components of cell surfaces and secreted molecules (24). Fucose, one of the nine core monosaccharides in glycoconjugates, is a consistent and informative marker for glycoprotein biosynthesis and secretion studies (17). The elevated levels of fucose observed in leukemia patients may be due to increased glycosyltransferase activity, loss of tumor-associated glycoproteins, and upregulation of enzymes like FUT8 (25, 26). This suggests that higher serum fucose levels could be a general indicator of oncologic activity, reflecting abnormal glycoprotein metabolism (27).

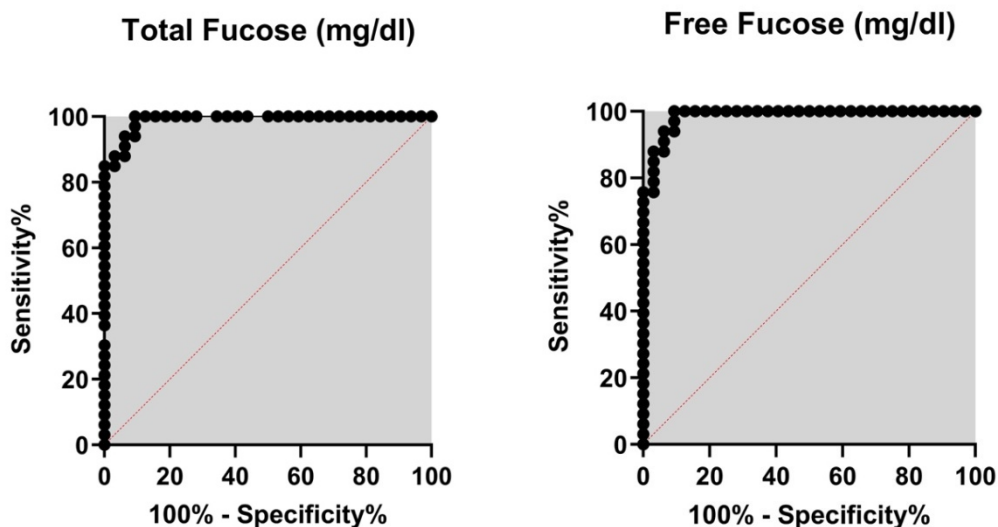


Figure 1. Represents the ROC curves for: a. Serum Total Fucose, b. Serum Free Fucose

The rise in FF may be linked to elevated activity of L-fucosidase, a lysosomal enzyme critical for fucose degradation and remodeling of glycosylation. Increased L-fucosidase activity has also been noted in other malignancies, such as oral cancer (24).

The observed decrease in PBH, despite elevated fucose levels, may point toward a metabolic shift favoring fucosylation over hexosylation during leukemic transformation. This supports the theory of competitive glycosylation remodeling in cancer, where specific pathways are upregulated at the expense of others (30).

The increase in serum fucose levels found in this study is consistent with previous reports in various cancers, including breast, oral, ovarian, and lung malignancies (25, 26). Higher PBF levels observed in our leukemia patients parallel findings in ovarian cancer cases before therapy initiation (27), suggesting that elevated fucosylation may be a marker of disease presence prior to treatment. Additionally, increased LAF has also been associated with tumors, supporting its potential diagnostic role in hematological malignancies (28). Conversely, the reduction in PBH contrasts with its elevation in ovarian cancer (23), suggesting disease-specific glycosylation signatures.

This study has several limitations. First, the small sample size limits the statistical power and generalizability of

the findings. Second, only male participants were included, precluding any assessment of gender differences. Lastly, potential confounding variables were not controlled in the statistical analysis, which may have influenced the results.

The high AUC values seen for TF and FF in the ROC curve analysis suggest promising diagnostic performance, potentially surpassing traditional tumor markers like CEA in colorectal cancer (29). If validated in larger cohorts, serum fucose and its derivatives could serve as useful non-invasive biomarkers for the early detection and monitoring of treatment in leukemia. These findings also align with earlier studies linking altered fucose metabolism to other systemic diseases such as myocardial infarction, diabetes, atherosclerosis, and cirrhosis (30), reinforcing its potential as a broad-spectrum biomarker. Future research should include larger, diverse populations, multivariate analyses to adjust for confounding variables, and longitudinal follow-up to assess prognostic and therapeutic implications.

Conclusion

This study shows that newly diagnosed male leukaemia patients have significantly higher levels of serum fucose and its variations, especially total and free fucose, than healthy controls. These changes in fucosylation patterns highlight their diagnostic utility and

raise the possibility of a role in leukaemia pathogenesis.

Both total and free fucose had good sensitivity and specificity according to Receiver Operating Characteristic (ROC) analysis, suggesting their value as non-invasive diagnostic biomarkers. Nevertheless, the results are constrained by the gender restriction and sample size. To confirm the diagnostic value and investigate the prognostic relevance of these fucose-related markers in leukaemia, future research involving larger, more varied populations and longitudinal follow-up is advised.

Competing interest

The authors declare that they have no competing interests.

References

1. Nemkov T, D'Alessandro A, Reisz JA. Metabolic underpinnings of leukemia pathology and treatment. *Cancer Rep (Hoboken)*. 2019;2(2):1139. <https://doi.org/10.1002/cnr2.1139>
2. Fernandes A, Shanmuganathan N, Branford S. Genomic mechanisms influencing outcome in chronic myeloid leukemia. *Cancers (Basel)*. 2022;14(3):620. <https://doi.org/10.3390/cancers14030620>
3. Athale U, Hijiya N, Patterson BC, Bergsagel J, Andolina JR, Bittencourt H, et al. Management of chronic myeloid leukemia in children and adolescents: Recommendations from the Children's Oncology Group CML Working Group. *Pediatr Blood Cancer*. 2019;66(9):27827. <https://doi.org/10.1002/pbc.27827>
4. Brown PA. Neonatal leukemia. *Clin Perinatol*. 2021;48(1):15-33. <https://doi.org/10.1016/j.clp.2020.11.002>
5. Malard F, Mohty M. Acute lymphoblastic leukaemia. *Lancet*. 2020;395(10230):1146-62. [https://doi.org/10.1016/S0140-6736\(19\)33018-1](https://doi.org/10.1016/S0140-6736(19)33018-1)
6. Grimwade D, Ivey A, Huntly BJ. Molecular landscape of acute myeloid leukemia in younger adults and its clinical relevance. *Blood*. 2016;127(1):29-41. <https://doi.org/10.1182/blood-2015-07-604496>
7. Hallek M, Shanafelt TD, Eichhorst B. Chronic lymphocytic leukaemia. *Lancet*. 2018;391(10129):1524-37. [https://doi.org/10.1016/S0140-6736\(18\)30422-7](https://doi.org/10.1016/S0140-6736(18)30422-7)
8. Newburg DS, Morelli L. Human milk and infant intestinal mucosal glycans guide succession of the neonatal intestinal microbiota. *Pediatr Res*. 2015;77(1):115-20. <https://doi.org/10.1038/pr.2014.178>
9. Pekdemir B, Karav S. Exploring the diverse biological significance and roles of fucosylated oligosaccharides. *Front Mol Biosci*. 2024;11:1403727. <https://doi.org/10.3389/fmolb.2024.1403727>
10. Bunyatratkata A, Le Parc A, de Moura JM, Cohen JL, Duman H, Arslan A, et al. Release of bifidogenic N-glycans

<https://doi.org/10.15218/zjms.2026.013>

- from native bovine colostrum proteins by an endo- β -N-acetylglucosaminidase. *Enzyme Microb Technol.* 2023;162:110138. <https://doi.org/10.1016/j.enzmictec.2022.110138>
11. Terao N, Takamatsu S, Minehira T, Sobajima T, Nakayama K, Kamada Y, et al. Fucosylation is a common glycosylation type in pancreatic cancer stem cell-like phenotypes. *World J Gastroenterol.* 2015;21(13):3876. <https://doi.org/10.3748/wjg.v21.i13.3876>
12. Pinho SS, Alves I, Gaifem J, Rabinovich GA. Immune regulatory networks coordinated by glycans and glycan-binding proteins in autoimmunity and infection. *Cell Mol Immunol.* 2023;20(10):1101-13. <https://doi.org/10.1038/s41423-023-01074-1>
13. Miwa N. Protein-carbohydrate interaction between sperm and the egg-coating envelope and its regulation by dicalcin, a *Xenopus laevis* zona pellucida protein-associated protein. *Molecules.* 2015;20(5):9468-86. <https://doi.org/10.3390/molecules20059468>
14. Li J, Hsu HC, Mountz JD, Allen JG. Unmasking fucosylation: from cell adhesion to immune system regulation and diseases. *Cell Chem Biol.* 2018;25(5):499-512. <https://doi.org/10.1016/j.chembiol.2018.02.005>
15. Lin B, Qing X, Liao J, Zhuo K. Role of protein glycosylation in host-pathogen interaction. *Cells.* 2020;9(4):1022. <https://doi.org/10.3390/cells9041022>
16. Pinho SS, Reis CA. Glycosylation in cancer: mechanisms and clinical implications. *Nat Rev Cancer.* 2015;15(9):540-55. <https://doi.org/10.1038/nrc3982>
17. Schneider M, Al-Shareffi E, Haltiwanger RS. Biological functions of fucose in mammals. *Glycobiology.* 2017;27(7):601-18. <https://doi.org/10.1093/glycob/cwx034>
18. Li Q, Wang X, Li X, He X, Wan Q, Yin J, et al. Obtaining high-quality blood specimens for downstream applications: a review of current knowledge and best practices. *Biopreserv Biobank.* 2018;16(6):411-8. <https://doi.org/10.1089/bio.2018.0052>
19. Mercatali L, Serra P, Misericocchi G, Spadazzi C, Liverani C, De Vita A, et al. Dried blood and serum spots as a useful tool for sample storage to evaluate cancer biomarkers. *J Vis Exp.* 2018;(136):57113. <https://doi.org/10.3791/57113>
20. Yaseen NA, Ahmed BM. Effect of cigarette smoking on serum α -L-fucose and its related parameters. *Zanco J Med Sci.* 2014;18(1):596-603. <http://dx.doi.org/10.15218/zjms.2014.0002>
21. Wang Y, Zhang X, Tian X, Wang Y, Xing X, Song S. Research progress on the functions, preparation and detection methods of l-fucose. *Food Chem.* 2024;433:137393. <https://doi.org/10.1016/j.foodchem.2023.137393>

<https://doi.org/10.15218/zjms.2026.013>

22. Gab-Allah MA, Kim J. A Comprehensive Review of Recent Advances in the Enrichment and Mass Spectrometric Analysis of Glycoproteins and Glycopeptides in Complex Biological Matrices. *Mass Spectrom Lett.* 2024;15(1):1-25.
<https://doi.org/10.5478/MSL.2024.15.1.1>
23. Wsoo MA, Ahmed BM. Evaluation of salivary α -L-Fucose and its related parameters in periodontitis. *Zanco J Med Sci.* 2013;17(3):563-9.
<http://dx.doi.org/10.15218/zjms.2013.0049>
24. Fu J, Guo Q, Feng Y, Cheng P, Wu A. Dual role of fucosidase in cancers and its clinical potential. *J Cancer.* 2022;13(10):3121.
<https://doi.org/10.7150/jca.75840>
25. Sawke NG, Sawke GK. Serum fucose level in malignant diseases. *Indian J Cancer.* 2010;47(4):452-7.
<https://doi.org/10.4103/0019-509X.73549>
26. Chen CY, Jan YH, Juan YH, Yang CJ, Huang MS, Yu CJ, et al. Fucosyltransferase 8 as a functional regulator of nonsmall cell lung cancer. *Proc Natl Acad Sci U S A.* 2013;110(2):630-5.
<https://doi.org/10.1073/pnas.1220425110>
27. Thakkar V, Patel P, Prajapati N, Kaur R, Nandave M. Serum levels of glycoproteins are elevated in patients with ovarian cancer. *Indian J Clin Biochem.* 2014;29:345-50.
<https://doi.org/10.1007/s12291-013-0380-6>
28. Mahdi NR, Ahmed FS. Biochemical Estimation of Total Sialic Acid, Lipid-Bound Sialic Acid and Fucose in Serum Patients with Nasal and Paranasal Sinus Malignancies. *Iraqi J Med Sci.* 2021;19(2).
<https://doi.org/10.22578/IJMS.19.2.2>
29. Sharma M, Sharma E, Prabhu V, Pai VR, D'souza JM, Harish S, et al. Salivary L-fucose as a biomarker for oral potentially malignant disorders and oral cancer. *J Cancer Res Ther.* 2020;16(3):546-50.
https://doi.org/10.4103/jcrt.JCRT_552_17
30. Ali DR, Mahmoud TJ, Hassan HG. Evaluation of serum fucose and protein bound fucose on myocardial infarction patients in Erbil city. *Zanco J Med Sci.* 2012;16(1):40-4.
<https://doi.org/10.15218/zjms.2012.0007>