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Diagnostic utility of GFAP and RANKL in thalassemia: Insights into neurological and bone-related alterations

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β-thalassemia a major health challenge in many parts of the world, including the Middle East. This study aimed to explore the diagnostic relevance of two biochemical markers (GFAP and RANKL) in thalassemia patients. This cross-sectional study was conducted from January to April 2024 at the International Center for Research and Development Laboratories. A total of 90 participants were enrolled, including 66 patients with confirmed β-thalassemia and 24 age- and sex-matched healthy controls. Eligibility criteria for patients included diagnosis based on hematological and genetic tests, age above 18, and absence of comorbidities. Individuals with acute infections, recent blood transfusions, or autoimmune/inflammatory diseases were excluded. Venous blood was collected, and serum GFAP and RANKL levels were measured using ELISA kits (Elabscience, China). Data were analyzed in SPSS v25 using independent t-tests. ROC analysis assessed diagnostic performance, while heatmap and PCA were used to examine biomarker relationships and clustering. Patients with thalassemia had significantly elevated serum levels of GFAP and RANKL. ROC curve analysis demonstrated excellent diagnostic accuracy for both markers: GFAP yielded an AUC of 100%, while RANKL showed an AUC of 99%. A strong positive correlation between the two biomarkers was observed in patients. PCA further confirmed a distinct biochemical profile in thalassemia, with both markers contributing prominently to group separation. The findings suggest that GFAP and RANKL may serve as promising biomarkers for detecting neurological and bone-related alterations in thalassemia. Their diagnostic potential supports further investigation into their role in disease monitoring and management.

Keywords: β-thalassemia; GFAP; RANKL; biomarkers; neurological complications; skeletal complications.

Introduction

Thalassemia is an inherited blood disorder caused by defective hemoglobin synthesis, leading to chronic anemia and multiple clinical complications. Among its types, β-thalassemia is the most common form, often requiring lifelong blood transfusions. Despite medical advances, issues such as iron overload and organ damage remain significant challenges (Su et al., 2025).

β-thalassemia is known as the most prevalent autosomal recessive hemoglobinopathy, caused by mutations in the β-globin gene located on chromosome 11. Globally, about 60,000 newborns are diagnosed annually with β-thalassemia major, with the majority residing in low- and middle-income countries. Approximately 1.5% of the world's population (around 80 to 90 million people) carry the genetic mutation for this disease (Rao et al., 2024; Hartman & Gallicchio, 2025). The condition is especially widespread in regions such as the Mediterranean basin, the Middle East, and parts of South and South-East Asia. In some Middle Eastern countries like Iraq, Iran, and Saudi Arabia, carrier rates can exceed 20% in certain population groups. Particularly in Iraq, β-thalassemia continues to be a major public health concern, exacerbated by high rates of consanguineous marriages and limited nationwide genetic screening programs (Rao et al., 2024).

Classified as a hemoglobinopathy, the clinical severity of β-thalassemia varies based on the underlying genetic and molecular defects. Treatment primarily relies on regular blood transfusions combined with iron chelation therapy; however, newer approaches like bone marrow transplantation and gene therapy are being investigated to achieve curative results (Su et al., 2025). Still, the long-term effects of chronic low oxygen levels and iron accumulation on different organs (including the nervous and skeletal systems) have not been fully explored. Recently, biochemical biomarkers have gained attention for their potential in understanding secondary complications related to thalassemia. One such marker is Glial Fibrillary Acidic Protein (GFAP), which is released mainly from astrocytes following neural injury or inflammation in the central nervous system. Elevated GFAP levels in the blood have been linked to conditions like stroke, traumatic brain injury, and neurodegenerative diseases (Heimfarth et al., 2022; Essa et al., 2024). Likewise, Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL) plays a key role in bone remodeling by promoting osteoclast differentiation and bone resorption. Increased RANKL expression is associated with bone loss and osteoporosis, common issues observed in thalassemia patients (Alfaqih et al., 2018).

In recent years, multiple studies have examined the role of various biochemical markers in predicting and monitoring thalassemia-related complications. Markers such as serum ferritin (reflecting iron overload) (Hadi et al., 2025), malondialdehyde (MDA) (reflecting oxidative stress) (Neaimy et al., 2024), and pro-inflammatory cytokines like TNF-α and IL-6 (Alayunt Nö et al., 2024), have been extensively studied. Although these markers provide valuable insights into iron metabolism, inflammation, and oxidative damage, they do not specifically target neurological or skeletal complications. In contrast, GFAP as a sensitive marker of central nervous system injury, and RANKL as a major regulator of bone resorption, offer focused information regarding thalassemia-related neurological dysfunction and bone deterioration. Studying these markers may help fill existing gaps in knowledge and provide a more comprehensive understanding of the systemic impacts of chronic thalassemia.

Given the vulnerability of thalassemia patients to systemic complications (especially neurological and bone-related) it is essential to investigate biomarkers like GFAP and RANKL in this population. Despite their clinical importance, few studies have evaluated these markers in thalassemia patients. Therefore, this study aims to measure serum levels of GFAP and RANKL in individuals with thalassemia compared to healthy controls, to shed light on the underlying pathophysiological mechanisms contributing to disease complications.

Materal and methods

This observational cross-sectional research was carried out between January and April 2024 at the Biochemistry Laboratory affilia-

ted with the International Center for Research and Development Laboratories. Prior to participation, all individuals provided written informed consent in accordance with ethical protocols.

A total of 90 subjects were recruited, comprising 66 individuals with clinically and genetically confirmed β-thalassemia and 24 age- and sex-matched healthy volunteers. Inclusion for the patient group required being over 18 years of age and free from chronic illnesses. Control participants had no documented history of hematologic or inflammatory conditions. Exclusion criteria for both groups included acute comorbidities, use of antibiotics, immunosuppressive or chemical drugs, and physiological states such as pregnancy, allergy, or recent physical strain. Individuals who had undergone blood transfusion within two weeks prior to sample collection were also excluded.

We used a sterile single- use 10mL syringe to draw 5 mL of venous blood from each participant under strict sterile conditions. After the samples were collected, they were transferred into simple tubes and left at room temperature to clot. Once the clot was formed, the samples were spun at 3,000 rpm to obtain serum. Then the serum was introduced into a large number of vials and kept at –20 ºC until it was used for biochemical assessment (Arif et al., 2025). We conducted analysis of Glial Fibrillary Acidic Protein (GFAP) and RANKL with the help of ELISA kits from Elabscience (China).The CASC procedures were all carried out according to the manufacturer's instructions. We tested every specimen in duplicate in the interests of accuracy. Absorbance readings were recorded at 450nm using a microplate reader from Thermo Scientific.

Data analysis was performed using IBM SPSS Statistics software version 25. Results for continuous variables are reported as mean ± standard error of the mean (SEM). The differences between the thalassemia and control groups were established by independent samples t-test at a significance level of $P < 0.05$. The receiver operating characteristic (ROC) curve analysis was conducted to evaluate the diagnostic ability of GFAP and RANKL In addition, we noted the area under the curve (AUC), sensitivity, specificity, and optimal threshold values. Patterns of correlation between biomarkers were distinguished using heatmap analysis. To examine further the differences between the groups, we subjected the dataset (Malik et al., 2023) to Principal Component Analysis (PCA) in order to determine the underlying structure of the variance and clustering.

Results

A total of 90 individuals participated in the study as a whole , including 66 people with thalassemia and 24 clinically healthy people. The average age between the two groups showed a significant difference, those with thalassemia averaging 54 years of age and the clinically healthy group averaging 47 years. Both groups had the same statistical average weight and there was no difference between the two groups in body mass index.

Table 1
T-test analysis of age, and BMI in two studied groups

Parameters		Thalassemia	Control	P-value
Age, year	mean ± standard error	54.1 ± 8.0	47.0 ± 9.9	0.0009
	lower 95% CI of mean	52.16	42.89	
	upper 95% CI of mean	56.11	51.28	
BMI	mean ± standard error	30.8 ± 6.9	29.5 ± 5.7	0.4119
	lower 95% CI of mean	29.10	27.09	
	upper 95% CI of mean	32.51	31.91	

Serum levels of GFAP were markedly elevated in thalassemia patients compared to healthy individuals (260 ± 30 vs. 123 ± 12; $P < 0.0001$). Similarly, the concentration of RANKL was significantly higher in the patient group (284 ± 43. vs. 130 ± 17; $P < 0.0001$).

Table 2
T-test analysis of GFAP, and RANKL in two studied groups

Parameters		Thalassemia	Control	P-value
GFAP	mean ± standard error	260 ± 30	123 ± 12	<0.0001
	lower 95% CI of mean	252.2	118.2	
	upper 95% CI of mean	267.1	128.7	
RANkL	mean ± standard error	284 ± 43	130 ± 17	<0.0001
	lower 95% CI of mean	273.4	122.7	
	upper 95% CI of mean	295.0	137.9	

As shown in Figures 1, the 95% confidence intervals indicate a statistically significant elevation in mean serum levels of RANKL and GFAP among thalassemia patients compared to healthy controls.

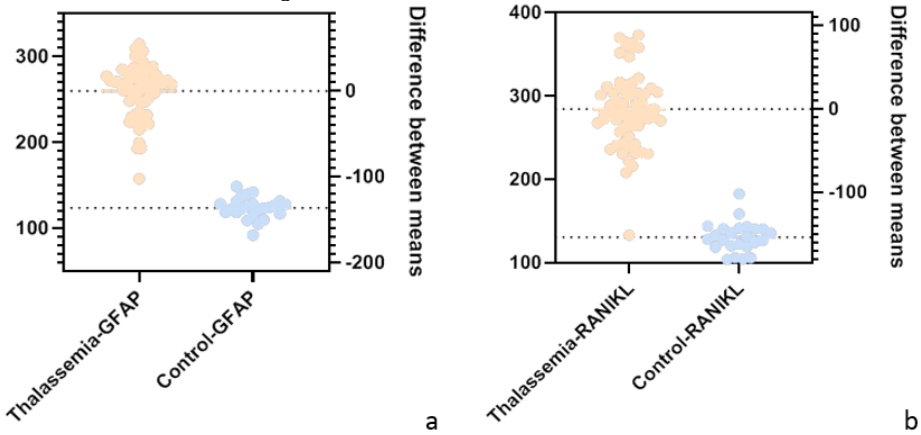

Fig. 1. 95% confidence intervals and the variation in group means for GFAP (*a*) and RANKL (*b*)

Receiver Operating Characteristic (ROC) curve analysis demonstrated that both GFAP and RANKL exhibited excellent diagnostic performance in distinguishing thalassemia patients from healthy controls. GFAP yielded an area under the curve (AUC) of 100%, with 100% sensitivity and 100% specificity at a cut-off value of 152.9. RANKL showed an AUC of 99%, with 98% sensitivity and 100% specificity at a cut-off value of 195.0.

Receiver Operating Characteristic (ROC) curves for GFAP and RANKL illustrate their diagnostic utility in identifying thalassemia patients. The high AUC values indicate excellent accuracy, sensitivity, and specificity for both biomarkers. Furthermore, principal component analysis (PCA) showed that the first two components accounted for 99% of the total variance in the data (PC1: 93.3%, PC2: 6.6%). The score plot demonstrated clear separation between patient and control groups, indicating a distinct biochemical profile. Loading analysis revealed that GFAP and RANKL contributed most strongly to the variance in PC1, suggesting that these biomarkers are key indicators of pathophysiological changes in thalassemia.

Table 3
ROC analysis of GFAP, and RANKL

Parameters	Area under the curve, %	Sensitivity, %	Specificity, %	Cutoff value
GFAP	100%	100%	100%	> 152.9
RANKL	99%	98%	100%	> 195.0

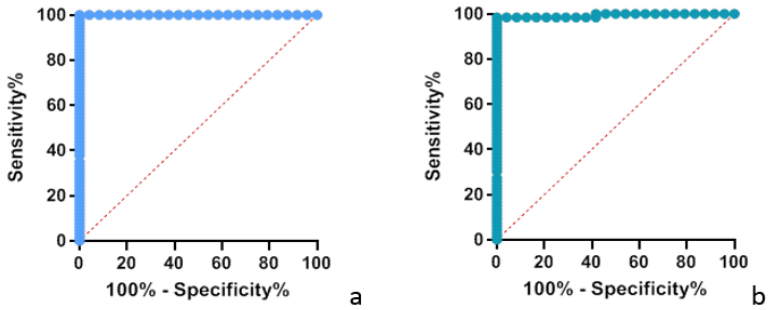

Fig. 2. ROC curve of GFAP (*a*) and RANKL (*b*): correlation analysis using heatmaps revealed a strong and significant positive correlation between GFAP and RANKL levels in both thalassemia and control groups, with a more pronounced relationship observed among the patients

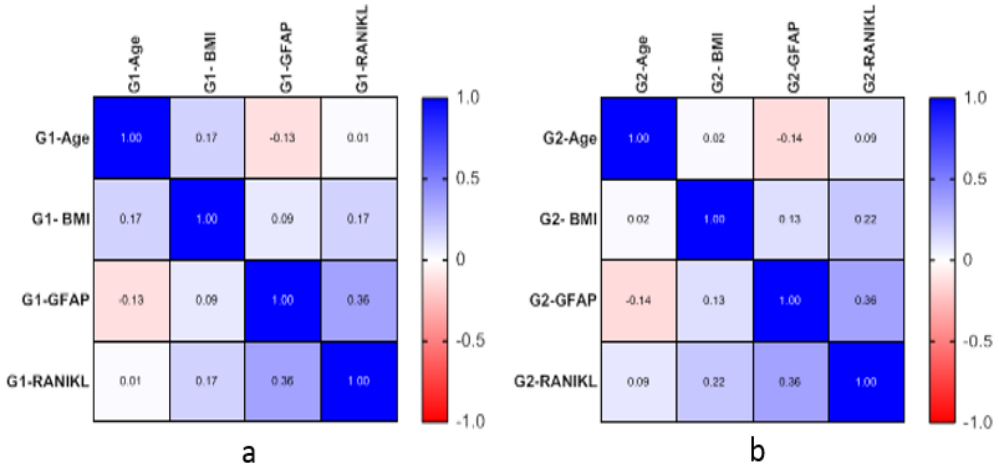

Fig. 3. Heatmap correlation matrices of GFAP and RANKL levels in thalassemia patients (*a*) and healthy controls (*b*): color intensity reflects the strength and direction of correlation between biomarkers

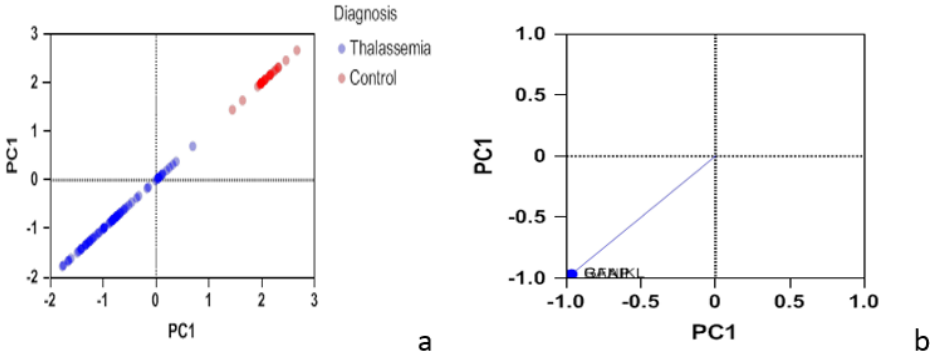

Fig. 4. Principal component analysis: score plot showing separation between thalassemia patients and controls along PC1 and PC2 (*a*); loading plot illustrating the strong contributions of GFAP and RANKL to PC1 (*b*)

Discussion

The research established that both GFAP and RANKL levels in the serum of thalassemia patients significantly exceeded the levels in the clinically healthy group. Diagnostic assessments, including ROC curve analysis and Principal Component Analysis (PCA), demonstrated that these biomarkers were highly accurate in separating patients from controls. Furthermore, correlation analysis with heatmap confirmed an association between both markers. The results acquired may well indicate systemic involvement of the nervous system and the skeletal system in the pathophysiology of thalassemia.

Similar results have been reported regarding RANKL, as in thalassemia patients, the expression of RANKL is raised or the RANKL/OPG ratio is changed, which indicates a rise in osteoclast activity and increase in bone resorption (Toumba & Skordis, 2010, Tsartsalis et al. 2019, Çelik et al. 2022). For example, Rashad et al. (2021) reported that raised serum RANKL levels were related to reduced bone mineral density (BMD) in a β-thalassemia major case, which confirms its role in bone disease (Rashad et al., 2021). Similarly, RANKL had an inverse relationship with BMD in adult thalassemia as per Zoga et al. (2024). With respect to the increase in GFAP levels, little is known about children with Sickle Cell Disease manifesting raised GFAP (Savage et al., 2011; Aydın et al., 2024). Considering that GFAP elevation in thalassemia is underresearched , our results provide new evidence for possible neuroinflammatory or neurodegenerative mechanisms present in these patients.

The increased levels of GFAP observed in thalassemia patients could indicate mild neural stress or damage on account of chronic hypoxia, oxidative stress or iron overload within the central nervous system (Pekny et al., 2004). When astrocytes are damaged, they respond by producing and releasing GFAP. We can detect this in the peripheral blood (Ozcelikay et al., 2024). Higher RANKL levels could also point to an imbalance in bone remodeling that favors resorption (Raje et al., 2019). Gaudio et al. (2019) and Hamidpour et al., (2022) point out that the biochemical modifications provide insights into some more subtle but nevertheless clinically significant complications of thalassemia apart from anemia and iron overload. In the research presented here, the diagnostic value and interrelation of GFAP and RANKL were determined statistically. The receiver operating characteristic curve is the most frequently used method of evaluating diagnostic accuracy with sensitivity and 1-specificity being plotted on the curve. The area under this curve (AUC) reveals the overall performance of the fitting (Zhou et al., 2011).

The ROC analysis of GFAP and RANKL had an AUC of 100% and 99%, respectively, showing a high ability to distinguish thalassemia patients from healthy people. Heatmaps are frequently employed in biomedical research – colours are used to indicate data, for example in correlation or clustering (Lane et al., 2020). According to the heatmap compiled in our research, GFAP has a strong positive correlation with RANKL, especially in the patient group. Possibly they share a common pathophysiological pathway due to inflammation, hypoxia, or iron overload. In addition, we applied principal component analysis (PCA) to our analysis. PCA is a dimensionality reduction technique. In this technique, first, correlated variables are transformed to uncorrelated principal components (Kurita et al., 2021). In our research, the results of the PCA revealed a clear distinction between thalassemia sufferers and healthy controls. In the PCA explanation, the loading plot of PC1 and PC2 indicated that first two components could explain 99% of total variance. The largest shares in GFAP and RANKL were shown in PC1 and thus are considered to possess biological significance in distinguishing the biochemical profiles of thalassemia. Therefore these two candidate biomarkers have the potential to be systemic markers.

The noticeable rise in GFAP and RANKL levels in thalassemia patients indicates that they could serve as early indicators of neurological and skeletal complications. Employing the tested metabolites in clinical follow-up seems promising on account of the strong diagnostic performance achieved in ROC analysis and the demarcation of groups effected by PCA. Possible future applications of our findings may involve routine biomarker screening in coordination with imaging studies such as DEXA scans or brain MRIs, which may improve timely detection of secondary complications in chronic thalassemia care.

The study has certain limitations. Due to its cross-sectional design, it is impossible to determine causal relationships. The size of the sample was limited while the absence of correlating clinical or imaging data (particularly neuroimaging or bone densitometry) detracts from the value of the biological findings. Future research should involve large and longitudinal cohorts and be evaluated through neurocognitive, radiological and skeletal measurements. Examination of therapeutic modulation of these markers, for example the effect of anti-resorptive agents or iron chelation on GFAP and RANKL would be of value.

Conclusion

The serum GFAP and RANKL levels were significantly higher in patients suffering from thalassemia in comparison to healthy individuals and this could play a role in the systemic complications of the disease. The two biomarkers showed an impressive diagnostic performance with high sensitivity and specificity. This argues for their use in identifying at-risk patients. A strong positive correlation was found between GFAP and RANKL. The clear separation of groups in PCA analysis shows that they can be complementary indicators of neuroinflammatory and skeletal changes in patients with thalassemia. These results could be used to design research aimed at integrating biochemical markers into future screening and monitoring of complications in thalassemia.

References

Alayunt, N. Ö., Yerlikaya, E., & Özüdoğru, O. (2024). Recent developments in patients with thalassemia: Comparison of antioxidant and cytokine levels and possible measures. Gevher Nesibe Journal of Medical and Health Sciences, 9(1), 136–142.

Alfaqih, M. A., Bashir, N., Saadeh, R., Khader, Y., Barqawi, M., & Alqudah, S. (2018). Dysregulation of the RANKL/RANK/OPG axis in thalassemia intermedia patients. BMC Research Notes, 11, 534.

Arif, A. I., Rmaidh, A. Y., Qaddoori, H. T., & Mohammad, S. Q. (2025). Importance of testosterone and cortisol in male patients with prediabetic and diabetic in Diyala Governorate (Iraq). Regulatory Mechanisms in Biosystems, 16(2), e25070.

Aydın, S., Çoban, Y., Akbaş, Y., Tunçer, G. Ö., Oktay, G., Yeral, H., Köker, A., Alparslan, A. Ş., & Ellidag, H. Y. (2024). S100B, GFAP and NSE: The role of neuro biomarkers for early predictors of cerebral vaso-occlusive manifestations of sickle cell disease. Neurochemical Journal, 18(3), 515–520.

Çelik, T., Sangün, Ö., Ünal, Ş., Balcı, A., & Motor, S. (2022). Assessment of biochemical bone markers of osteoporosis in children with thalassemia major. Italian Journal of Pediatrics, 48, 105.

Essa, S. H., Mohammad, S. Q., Kadhum, D. A., & Jalil, I. S. (2024). Effectiveness of silver nanoparticles as antibacterial agents with natural wound healing cream with extracted *Aloe vera* gel. Regulatory Mechanisms in Biosystems, 15(4), 821–825.

Gaudio, A., Morabito, N., Catalano, A., Rapisarda, R., Xourafa, A., & Lasco, A. (2019). Pathogenesis of thalassemia major-associated osteoporosis: A review with insights from clinical experience. Journal of Clinical Research in Pediatric Endocrinology, 11(2), 110–117.

Graf, M., & Gallicchio, V. S. (2022). History, etiology, and treatment of paroxysmal nocturnal hemoglobinuria. Trends in Internal Medicine, 2(1), 1–8.

Hadi, A. M., Yas, L. S., Saeed, S. A., & Al-Dulimi, A. G. (2025). Iron overload and serum and saliva ferritin levels in individuals with beta thalassemia needing several blood transfusions. Dentistry 3000, 13(1), a001.

Hamidpour, M., Jafari, F., Mehrpouri, M., Azarkyvan, A., Bashash, D., & Khadem Maboudi, A. A. (2022). Evaluation of relationship between biochemical parameters and osteoporosis in patients with β-thalassemia major. Iranian Journal of Pediatric Hematology and Oncology, 12(1), 8360.

Hartman, A. R., & Gallicchio, V. S. J. (2025). Treatment options for beta-thalassemia. Trends in Internal Medicine, 5(1), 1–7.

Heimfarth, L., Passos, F. R. S., Monteiro, B. S., Araújo, A. A. de S., Quintans Jr., L. J., & Quintans, J. de S. S. (2022). Serum glial fibrillary acidic protein is a body fluid biomarker: A valuable prognostic for neurological disease – A systematic review. International Immunopharmacology, 107, 108624.

Kurita, T. (2021). Principal component analysis (PCA). In: Ikeuchi, K. (Ed.). Computer Vision: A Reference Guide. Springer. Pp. 1013–1016.

Lane, M., Maiocco, A., Bhatia, S. K., & Climer, S. (2020). Eyeing the patterns: Data visualization using doubly-seriated color heatmaps. Advances in Computers, 119, 121–156.

Malik, A. W., Abood, A. A., & Mohammad, S. Q. (2023). Association between the proinflammatory cytokine IL-17F and *Helicobacter pylori* infection in a sample of Iraqi patients. International Journal of Biomedicine, 13(2), 338–341.

Neaimy, K. S. A., Alkhyatt, M. M., & Jarjess, I. A. (2024). New insights of oxidative stress and thalassemia may lead to antioxidant therapy. Pharmacognosy Journal, 16(1), 202–204.

Ozcelikay-Akyildiz, G., Karadurmus, L., Cetinkaya, A., Uludag, İ., Ozcan, B., Unal, M. A., Sezginturk, M. K., & Ozkan, S. A. (2024). The evaluation of clinical applications for the detection of the Alzheimer's disease biomarker GFAP. Critical Reviews in Analytical Chemistry, 2024, 2393874.

Pekny, M., & Pekna, M. (2004). Astrocyte intermediate filaments in CNS pathologies and regeneration. The Journal of Pathology, 204(4), 428–437.

Raje, N. S., Bhatta, S., & Terpos, E. (2019). Role of the RANK/RANKL pathway in multiple myeloma. Clinical Cancer Research, 25(1), 12–20.

Rao, E., Kumar Chandraker, S., Misha Singh, M., & Kumar, R. (2024). Global distribution of β-thalassemia mutations: An update. Gene, 896, 148022.

Rashad, N. M., El-Helaly, A. M., Radwan, A. M., & Ibrahim, N. F. (2021). Association of receptor activator of nuclear factor-κB ligand (RANKL) and osteoprotegerin with secondary hypogonadism in Egyptian females with beta-thalassemia major. The Egyptian Journal of Hospital Medicine, 82(2), 369–378.

Savage, W. J., Barron-Casella, E., Fu, Z., Dulloor, P., Williams, L., Crain, B. J., White, D. A., Jennings, J. M., Van Eyk, J. E., Debaun, M. R., Everett, A., & Casella, J. F. (2011). Plasma glial fibrillary acidic protein levels in children with sickle cell disease. American Journal of Hematology, 86(5), 427–429.

Su, Y., Xie, J., He, J., Shen, Y., Li, T., Huang, W., Tong, X., & Bian, Q. (2025). Screening and treatment of thalassemia. Clinica Chimica Acta, 570, 120211.

Toumba, M., & Skordis, N. (2010). Osteoporosis syndrome in thalassaemia major: An overview. Journal of Osteoporosis, 2010, 537673.

Tsartsalis, A. N., Lambrou, G. I., Tsartsalis, D. N., Papassotiriou, I., Vlachou, E., Terpos, E., Chrousos, G. P., Kanaka-Gantenbein, C., & Kattamis, A. (2019). Bone metabolism markers in thalassemia major-induced osteoporosis: Results from a cross-sectional observational study. Current Molecular Medicine, 19(5), 335–341.

Zhou, X.-H., Obuchowski, N. A., & McClish, D. K. (2011). Statistical methods in diagnostic medicine. Wiley Series in Probability and Statistics. John Wiley & Sons.

Zoga, J., Kallco, M., & Refatllari, E. (2024). Correlation of OPG/RANKL in patients with thalassemia major at the center of Haemoglobinopathy Lushnje, Albania. European Scientific Journal, 20(18), 13.