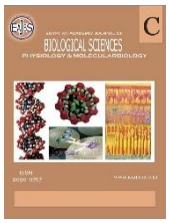
.

Citation: *Egypt.Acad.J.Biolog.Sci. (C.Physiology and Molecular biology) Vol. 16(1) pp121-128 (2024)*
DOI: 10.21608/EAJBSC.2024.339601

Egypt. Acad. J. Biolog. Sci., 16(1):123-128 (2024)

Egyptian Academic Journal of Biological Sciences
C. Physiology & Molecular Biology
ISSN 2090-0767
www.eajbsc.journals.ekb.eg



Evaluation of Midkine Protein in a Sample of Iraqi Patients with Newly Diagnosed Hypothyroidism and Subclinical Hypothyroidism

Mohammed k. Hussein[1] Mohammed I. Hamzah[2] and Mahmood S. Khudhair[3]
Department of Chemistry and Biochemistry ,College of Medicine, Al-Nahrain University,Baghdad, Iraq
***E-mail:** mkhjm8686@gmail.com

ARTICLE INFO
Article History
Received:28/12/2023
Accepted:2/2/2024
Available:6/2/2024

Keywords:
Hypothyroidism , Subclinical Hypothyroidism , Total T3, Total T4, TSH, Midkine , Na, k , Cl.

ABSTRACT

Thyroid hormone deficiency, with a variety of causes and symptoms, is known as hypothyroidism. Morbidity and mortality are increased by untreated hypothyroidism**.** Midkine is highly expressed during embryogenesis and governs cell proliferation, angiogenesis, anti-apoptotic actions, survival, and migration, though it is commonly low in maturity**.** Studies demonstrate that MK expression is highly elevated in thyroid cancers and connected to pathological and clinical characteristics, This study evaluates the relationship of MK with thyroid hormone levels in newly diagnosed hypothyroidism and subclinical hypothyroidism compared with healthy. a case-control study was conducted For the period from November 2022 to January 2023. The 120 participants in the current study, who were Baghdad residents between the ages of (20-65), were divided into three groups: 30 patients were newly diagnosed with hypothyroidism, and 30 patients had subclinical hypothyroidism. In contrast with 60 healthy subjects (control group). Measurements of S.TSH, Total S.T3, and Total S.T4 parameters. were measured on all groups and the results were compared between them by system automatically and (midkine) by ELISA and whole Electrolytes (Na, k, cl) were measured using a Manual spectrophotometer (colorimetric). The Results The midkine levels were measured and compared between the control group with the newly diagnosed hypothyroidism group, and the subclinical hypothyroidism group. There was a difference, but this difference did not reach statistical significance, there was a trend towards higher midkine levels in the NDH group compared to the SCH groups and control ($p=0.07$). while Statistical analysis indicated a significant difference in the midkine levels were measured and compared between the control group and the newly diagnosed hypothyroidism group ($p<0.04$). As for Age, weight, height and BMI However, these results were not statistically significant. (The probability value was greater than 0.05).

The control group had a mean sodium level of 145.32 ±6.90, the NDH group had a mean sodium level of 144.43 ±6.30, and the SCH group had a mean sodium level of 143.83 ±7.97. Statistical analysis did not show a significant difference in sodium levels among the groups $p=0.62$. while observing Statistical analysis revealed a significant difference in potassium levels among the groups ($p<0.001$). A pairwise comparison showed that the control group had higher potassium levels compared to both the NDH and SCH groups ($p<0.001$).

As for chloride, it was noted Statistical analysis indicated a significant difference in chloride levels among the groups ($p=0.01$). The pairwise comparison revealed that the SCH group had significantly lower chloride levels compared to both the control and NDH groups ($p<0.05$).

INTRODUCTION

Hypothyroidism results from low levels of thyroid hormone with varied etiology and manifestations. The drug of choice for the treatment of hypothyroidism is thyroid hormone replacement. Untreated hypothyroidism increases morbidity and mortality. This activity reviews the etiology, clinical presentation, diagnosis, and management of hypothyroidis (Patil, Rehman *et al.* 2021)**.** Midkine is highly expressed during embryogenesis and governs cell proliferation, angiogenesis, anti-apoptotic actions, survival, and migration, though it is commonly low in maturity (Ross-Munro, Kwa *et al*. 2020). Studies demonstrate that MK expression is highly elevated in thyroid cancers and is connected to pathological and clinical characteristics, indicating MK's promise as a thyroid cancer marker. Also, the accuracy, specificity, and diagnostic sensitivity rates for the identification of malignant and benign thyroid nodules were generated. However, it is present in more than 20 tumour diseases, requiring support from additional sources (Zhao, Zhang *et al.* 2021). This study is to determine MK levels in newly diagnosed hypothyroidism and subclinical hypothyroidism compared with healthy control to evaluate the relationship between MK and thyroid hormone levels.

MATERIALS AND METHODS

A case-control study was conducted at the Department of Chemistry and Biochemistry, College of Medicine, Al-Nahrain University. The specimens were collected from the "clinical biochemistry lab" at Imamein Kadhimein Medical City and the Specialized Center for Endocrinology & Diabetes/ Baghdad AL – Russafa, For the period from November 2022 to January 2023. The current study was conducted on 120 subjects chosen with an age range of (20-65 years) who was living in Baghdad, these subjects were divided into three groups, such as 30 Patients with newly diagnosed hypothyroidism (6 Males and 24 Females) and 30 Patients with subclinical hypothyroidism (6 Males and 24 Females) Compared with 60 healthy individuals (control group samples) 48 were females,12 males. were tested in a lab to determine if their serum TSH, total T4, and total T3 levels matched the typical reference range for thyroid hormone variations.

A clinical and laboratory examination was conducted. Total S.T3, Total S.T4, and S.TSH parameters were measured as a portion of a laboratory investigation. were measured on all groups and the results were compared between them by system automatically and (midkine) by ELISA and whole Electrolytes (Na, k, cl) were measured using a Manual spectrophotometer (colorimetric).

RESULTS

The Demographic characteristics of patients and control subjects are shown in Table 1.The sex distribution among the study groups showed no significant differences in association between sex and the groups (control, NDH, SCH). The test yielded a chi-squared value of 0.161, p-value was calculated to be 0.9225, indicating no significant association between sex and the groups. The majority of participants were female, comprising approximately 80.8% of the total population. These findings suggest that sex is not a confounding factor in the evaluation of midkine and thyroid hormone levels in patients with newly diagnosed hypothyroidism and subclinical hypothyroidism, As for About age, weight, height and BMI However, these results were not statistically significant. (The probability value was greater than 0.05).

The levels of sodium (Na), potassium (K), and chloride CL were measured and compared between the control group, the newly diagnosed hypothyroidism group, and the subclinical hypothyroidism group are shown in Table 2.The control group had a mean sodium level of 145.32 ±6.90, the NDH group had a mean sodium level of 144.43 ±6.30, and the SCH group had a mean sodium level of 143.83 ±7.97. Statistical analysis did not show a significant difference in sodium levels among the groups p=0.62.

while observing Statistical analysis revealed a significant difference in potassium levels among the groups ($p<0.001$). A pairwise comparison showed that the control group had higher potassium levels compared to both the NDH and SCH groups ($p<0.001$).

As for chloride, it was noted Statistical analysis indicated a significant difference in chloride levels among the groups ($p=0.01$). The pairwise comparison revealed that the SCH group had significantly lower chloride levels compared to both the control and NDH groups ($p<0.05$).

The midkine levels were measured and compared between the control group, the newly diagnosed hypothyroidism group, and the subclinical hypothyroidism group. There was a significant difference, but this difference did not reach statistical significance, there was a trend towards higher midkine levels in the NDH group compared to the control and SCH groups ($p=0.07$). Statistical analysis indicated a significant difference in the midkine levels were measured and compared between the control group and the newly diagnosed hypothyroidism group ($p<0.04$) This is likely to be a predictive marker for diagnosis of hypothyroidism or monitoring the progress complication disease demonstrated in Table 3.

Based on these observations, the diagnostic potential of these markers was assessed using receiver operator characteristic (ROC) curve analysis in order to find the best cutoff value of these markers that can predict a diagnosis of sensitivity and specificity and the results were shown in Table 4.

Midkine: The AUC is 0.593, indicating low discriminative ability. The optimal cutoff value is >546.8. Sensitivity is 76.67%, specificity is 53.33%, and the +LR is 1.64. The -LR is 0.44. These findings suggest that Midkine levels have a limited ability to differentiate between NDH and Control groups. Midkine: The AUC is 0.511, indicating poor discriminative ability. The optimal cutoff value is >479.05. Sensitivity is 93.33%, specificity is 30.00%, and the +LR is 1.33. The -LR is 0.22. These findings suggest that Midkine levels have a limited ability to differentiate between SCH and Control groups.

Table 1 Demographic and Clinical Characteristics of the Study Groups.

Parameter	Subjects	n	Median	Mean± SD	SEM	NDH	SCH	Control	P-value
Age years	Control	60	45.50	44.60± 11.23	2.05	A	A	A	0.12
	NDH	30	48.50	45.87± 11.49	2.10				
	SCH	30	35.00	39.87± 12.71	2.32				
Wt kg	Control	60	78.25	85.16± 18.52	2.39	A	A	A	0.09
	NDH	30	89.00	87.39± 14.91	2.72				
	SCH	30	94.50	93.99± 18.71	3.42				
Ht cm	Control	60	157.00	160.12± 9.39	1.21	A	A	A	0.29
	NDH	30	159.00	161.02± 6.71	1.22				
	SCH	30	161.50	163.15± 8.57	1.56				
BMI kg/m2	Control	60	32.17	33.19± 6.78	0.88	A	A	A	0.41
	NDH	30	34.65	33.84± 6.44	1.18				
	SCH	30	35.75	35.09± 5.11	0.93				

NDH= newly diagnosed hypothyroidism, SCH= subclinical hypothyroidism SEM= Standard error of the mean, groups having letters in common are statistically no different
*P values of one-way ANOVA

Table 2: Serum Electrolyte Levels with ANOVA Comparison Among the Groups.

Parameter	Subjects	n	Median	Mean± SD	SEM	NDH	SCH	Control	P *values*
Na mmol/L	Control	60	144.00	145.32± 6.90	0.89	A	A	A	0.62
	NDH	30	144.00	144.43± 6.30	1.15				
	SCH	30	144.00	143.83± 7.97	1.46				
K mmol/L	Control	60	4.50	4.38± 0.49	0.06	AB	B	A	<0.001*
	NDH	30	4.00	3.94± 0.41	0.07				
	SCH	30	4.00	3.91± 0.57	0.10				
Cl mEq/L	Control	60	100.00	100.98± 2.94	0.38	AB	B	A	0.01*
	NDH	30	100.00	100.60± 2.31	0.42				
	SCH	30	99.00	99.17± 1.53	0.28				

NDH= newly diagnosed hypothyroidism, SCH= subclinical hypothyroidism SEM= Standard error of the mean, groups having letters in common are statistically no different
*P values of one-way ANOVA
* Significant

Table 3: Midkine Descriptive Statistics with ANOVA Comparison Among the Groups.

Parameter	Subjects	n	Median	Mean± SD	SEM	NDH	SCH	Control	P value*
Midkine pg/ml	Control	60	545.77	567.71± 112.33	14.50	A	A	A	0.07
	NDH	30	589.15	640.05± 225.55	41.18				
	SCH	30	540.46	572.85± 94.67	17.28				

NDH= newly diagnosed hypothyroidism, SCH= subclinical hypothyroidism SEM= Standard error of the mean, groups having letters in common are statistically no different
*P values of one-way ANOVA
* Significant

Table 4 : Receiver Operating Characteristic (ROC) analysis, comparing different combinations of groups (NDH, SCH and control) using the markers Midkine.

contrasts	markers	AUC	SE	95% CI	Cutoff	Sens.	Spec.	+LR	-LR
NDH vs Control	Midkine	0.593	0.0618	0.485 to 0.696	>546.8	76.67	53.33	1.64	0.44
SCH vs Control	Midkine	0.511	0.0617	0.403 to 0.618	>479.05	93.33	30.00	1.33	0.22
NDH vs SCH	Midkine	0.597	0.0755	0.462 to 0.721	>540.80	76.67	53.33	1.64	0.44

SE: Standard Error, 95% CI: 95% Confidence, Interval, AUC: Area Under the Curve Cutoff: Threshold or Cut-off value, Sens.: Spec.: Specificity, Sensitivity,, -LR: Negative Likelihood Ratio, +LR: Positive Likelihood Ratio

DISCUSSION

The midkine levels were measured and compared between the control group, the newly diagnosed hypothyroidism group, and the subclinical hypothyroidism group. There was a difference, but this difference did not reach statistical significance, there was a trend towards higher midkine levels in the NDH group compared to the control and SCH groups (p=0.07). Mean values and pairwise comparison between groups.

Statistical analysis indicated a significant difference in the midkine levels were measured and compared between the control group and the newly diagnosed hypothyroidism group ($p<0.04$) This is likely to be a predictive marker for diagnosis of hypothyroidism or monitoring the progress complication disease.

The current findings are in line with the findings of numerous prior research, which found that midkine level concentration was considerably higher in the NDH group

compared to the control (Jawad M & Hussein,A..H. 2022). and from complications for hypothyroidism-related cardiovascular disease, MK has been associated with a number of cardiovascular diseases, and many research investigations on cardiovascular diseases have found elevated MK expression. (Majaj and Weckbach 2022)**.**Estimating serum electrolyte levels may be essential to controlling hypothyroid patients; it should be taken into account as it can assist in preventing future issues (Koner and Chaudhuri 2022). Statistical analysis did not show a significant difference in sodium levels among the groups, This result matched past studies (Wiwanitkit 2020).

A pairwise comparison showed that the control group had higher potassium levels compared to both the NDH and SCH groups ($p<0.001$). Mean values and pairwise comparison between groups and this result was in agreement with other studies (Singh, Dinkar *et al.* 2023). and Additionally (Kavitha, Pujar *et al*. 2017) As for chloride, it was noted Statistical analysis indicated a significant difference in chloride levels among the groups ($p=0.01$). The pairwise comparison revealed that the SCH group had significantly lower chloride levels compared to both the control and NDH groups ($p<0.05$). The current findings are in line with the findings of numerous prior research, which found that chloride levels concentration was considerably lower in hypothyroidism compared to the control (Sunita Pujar 2018).

Midkine levels showed a moderate positive correlation with TSH levels ($r = 0.27$). indicating a potential relationship between midkine and thyroid hormone regulation in the NDH group.

Midkine levels showed a moderate negative correlation with Na levels ($r = -0.40$) and Midkine levels showed a weak negative correlation with k levels ($r = -0.16$). Midkine levels showed a weak positive correlation with Cl levels ($r = 0.08$). indicating potential relationships between Midkine and electrolyte balance in the NDH group. Midkine levels showed a weak positive correlation with TSH levels ($r = 0.09$). indicating a potential relationship between midkine and thyroid hormone regulation in the SCH group. Midkine levels showed a weak positive correlation with Na levels ($r = 0.22$) and Midkine levels showed a moderate negative correlation with k levels ($r = -0.27$). Midkine levels showed a weak negative correlation with Cl levels ($r = -0.07$) indicating potential relationships between Midkine and electrolyte balance in the SCH group.

Conclusion:

1- Consider midkine as an early sign and likely to play a diagnostic role and early detection of complications in patients with newly diagnosed hypothyroidism (NDH).

2- There is no effect of the change in sex, age, weight, Height, body mass index of hypothyroidism, and subclinical hypothyroidism on the levels of midkine.

Declarations:

Ethical Approval:Ethical Approval is applicable.

Conflict of interests: The authors declare no conflict of interest.

Contributions:I hereby verify that all authors mentioned on the title page have made substantial contributions to the conception and design of the study, have thoroughly reviewed the manuscript, confirm the accuracy and authenticity of the data and its interpretation, and consent to its submission.

Funding: No funding was received.

Availability of Data and Materials: All datasets analysed and described during the present study are available from the corresponding author upon reasonable request.

REFERENCES

Jawad, M. M., & Hussein, A. H. (2022). Study of midkine protein as prognostic marker for thyroid disease in Al-Najaf City. *International Journal of Health Sciences*, 6(S5), 10990–11001.

Kavitha, M., Pujar, S., Hiremath, C., & Prasad, S. (2017). Evaluation of serum electrolytes in hypothyroid patients. *Med Pulse*, 1, 393-395.

Koner, S., & Chaudhuri, A. (2022). A study to evaluate the outcomes of hypothyroidism on serum potassium levels in an urban female population of Eastern India. *Current Medical Issues*, 20(4), 235-239.

Majaj, M., & Weckbach, L. T. (2022). Midkine A novel player in cardiovascular diseases. *Frontiers in Cardiovascular Medicine*, 9: 1003104.doi: 10.3389/fcvm. 2022. 1003104

Patil, N., Rehman, A., Jialal, I., & Saathoff, A. D. (2021). Hypothyroidism (Nursing). In:. StatPearls Publishing ,*Treasure Island (FL);* 2023. PMID: 33760505.

Ross-Munro, E., Kwa, F., Kreiner, J., Khore, M., Miller, S. L., Tolcos, M.,Walker, D. W. (2020). Midkine: the who, what, where, and when of a promising neurotrophic therapy for perinatal brain injury. *Frontiers in neurology*, 1284.

Singh, J., Dinkar, A., Kumar, N., & Kumar, K. (2023). Recurrent hypokalemic paralysis in hypothyroidism. *The American journal of the medical sciences.*Volume 365, Issue 5,

Sunita Pujar, Kavitha, M, Hiremath, C., & Prasad, S. (2018). Evaluation of serum electrolytes in hypothyroid patients. *Med Pulse*, 1, 393-395.

Wiwanitkit, V. (2020). Hyponatremia and hypothyroidism. *Medical Journal of Dr. DY Patil University*, 13(3), 242-243.

Zhao, Z.-W., Zhang, M., Liao, L.-X., Zou, J., Wang, G., Wan, X.-J.,Yu, X.-H. (2021). Long non-coding RNA PCA3 inhibits lipid accumulation and atherosclerosis through the miR-140-5p/RFX7/ABCA1 axis. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*, 1866(5), 158904.