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Research Article

Evaluation of Metanephrine and Lactate Dehydrogenase in Pediatric Wilms Tumor and Neuroblastoma

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Abstract

Background: Wilms' tumor and Neuroblastoma are common pediatric malignancies, with biochemical markers like plasma metanephrines and serum lactate dehydrogenase (LDH) being studied for diagnostic and prognostic values. Metanephrine, a catecholamine metabolite, is elevated in Neuroblastoma due to tumor secretion. Elevated LDH levels may correlate with tumor burden and indirectly indicate disease activity.

Objectives: To evaluate plasma metanephrine and lactate dehydrogenase as biomarkers in Neuroblastoma and Wilms tumor in pediatric patients.

Methods: The study was conducted between February and December 2024, in the Children Welfare Teaching Hospital, Medical City Complex, Baghdad, Iraq, and the Department of Biochemistry, College of Medicine, University of Baghdad. A Total of 98 children under the age of 10 years were included and grouped into: Group 1: 34 patients with Neuroblastoma, Group 2: 31 patients with Wilms tumor, and Group 3: 33 healthy children serving as controls. Blood samples (5 mL) were collected from all participants. Plasma metanephrine levels were measured using an ELISA method, while lactate dehydrogenase levels were determined using the Roche Cobas analyzer. Anthropometric parameters were also measured.

Results: The study found no significant differences in age, weight, or height among Neuroblastoma, Wilms tumor, and control groups. However, Neuroblastoma patients showed significantly higher Metanephrine levels (26.2 ± 2.24 ng/mL) compared to Wilms tumor (8.0 ± 0.28 ng/mL) and controls (7.5 ± 0.29 ng/mL) . Similarly, LDH levels were elevated in Neuroblastoma (848.8 ± 87.57 U/L) and Wilms tumor (629.3 ± 66.99 U/L) versus controls (68.6 ± 3.90 U/L), with Neuroblastoma exhibiting higher LDH than Wilms tumor.

Conclusion: The study emphasizes the importance of using metanephrine measurements for Neuroblastoma diagnosis and monitoring, as these metabolites can inform treatment response and disease progression. Neuroblastoma exhibits higher levels of metanephrine and lactate dehydrogenase, indicating a larger tumor burden. Wilms tumor does not share these characteristics.

Keywords: Lactate Dehydrogenase; Metanephrine; Neuroblastoma; Pediatric cancer; Wilms tumor.

Introduction:

Neuroblastoma is the most common extracranial solid tumor of childhood and the most prevalent in the first year of life (1). The tumor can occur anywhere in the body but is most commonly located in the abdominal area, particularly in the adrenal glands, and may spread to the spine (2). The symptoms of Neuroblastoma depend on the sites of metastasis. Neuroblastoma typically affects children under the age of 5 and is rare in those over 10 years old, although it can occasionally present in older children and adults (3). Wilms tumor, or nephroblastoma, is the most common renal malignancy in children, named after the German surgeon Max Wilms, who first described it in 1899 (4). The incidence of Wilms tumor is approximately 5.4 cases per 1,000,000 children and adolescents. Female sex and age under 5 years at diagnosis are associated with a higher incidence of Wilms tumor (5). The clinical presentation of Wilms tumor typically includes a palpable abdominal mass, potential hematuria, and various less common symptoms (6).

Metanephrine is a metabolite of norepinephrine, formed through a biochemical pathway that begins with the amino acid tyrosine (7). This pathway involves several steps: Tyrosine is converted to Dihydroxyphenylalanine, which is then converted to dopamine, followed by norepinephrine, and finally to metanephrine through the action of the enzyme catechol-O-methyltransferase (COMT). The molecular

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structure of metanephrine includes a catechol group and a methylated amino side chain, distinguishing it from norepinephrine. This structure influences its reactivity and activity at adrenergic receptors (8).

In the body, metanephrine plays a minor role in the 'fight or flight' response but mainly functions as a diagnostic marker. Elevated levels of metanephrine in blood or urine can indicate certain diseases or tumors that produce excess catecholamines (9). Therefore, measuring metanephrine is crucial for diagnosing these tumors, highlighting its significance in clinical diagnostics and the adrenergic system, especially in conditions like pheochromocytomas and paragangliomas (10). The clinical importance of plasma catecholamines in pediatric cancer, particularly neuroblastoma, lies in their role as diagnostic and prognostic biomarkers. Elevated levels of catecholamines and their metabolites, such as, Dihydroxyphenylalanine (DOPA), Dihydroxyphenylacetic acid (DOPAC), Homovanillic acid (HVA), and Vanillylmandelic acid (VMA), suggest neuroblastoma and help monitor disease progression and treatment response. Neuroblastoma, a tumor that almost exclusively affects children, originates from immature embryonic neuroblasts and forms tumors in intra-adrenal and extra-adrenal locations. Underdeveloped catecholamine biosynthetic and secretory pathways characterize neuroblastoma, although variations influence biochemical testing and disease severity (11).

Lactate Dehydrogenase is an enzyme crucial for converting lactate to pyruvic acid, a key step in cellular energy production, particularly under anaerobic conditions (12). It plays a critical role in energy metabolism and serves as a biomarker for various medical conditions, including tissue damage and certain malignancies. Lactate Dehydrogenase exists as five isoenzymes, formed by combinations of two subunits: LDH-A (M) and LDH-B (H), encoded by genes located on chromosomes 11 and 12, respectively (13).

Under anaerobic conditions, LDH catalyzes the reverse reaction, converting lactate to pyruvate, a vital process during intense exercise when oxygen availability is limited. This reaction is integral to the Cori cycle, which helps regulate blood lactate levels and facilitates lactate recycling for energy production in the liver. This metabolic flexibility underscores LDH role in maintaining cellular energy balance (14). Elevated LDH levels in pediatric cancer patients serve as a significant biomarker for disease progression and treatment outcomes. High LDH levels are often indicative of tumor burden and correlate with poor prognosis, making them a valuable tool in clinical practice for monitoring treatment efficacy and disease status (15).

Aim of study: To evaluate plasma metanephrine and lactate dehydrogenase as biomarkers in Neuroblastoma and Wilms tumor in pediatric patients.

Subjectes and Methods:

This case-control study was conducted at the Children Welfare Teaching Hospital, Medical City Complex, Baghdad, Iraq, and by the Department of Biochemistry, College of Medicine, University of Baghdad, from February to December 2024. The study included 98 pediatric subjects under the age of 10 years. The case group included patients diagnosed with solid tumors, specifically Neuroblastoma and Wilms tumors, under active chemotherapy, and a control group of healthy children who were eligible children under 10 years of age, with no prior diagnosis of chronic illnesses, and were enrolled after obtaining written informed consent from their parents. The study population was grouped into: Group I: 34 subjects with Neuroblastoma; Group II: 31 subjects with Wilms tumor; and Group III: 33 healthy children (control group).

Ethical approval was obtained from the scientific and ethical committees of the Department of Biochemistry / College of Medicine / University of Baghdad, as well as from the Children Welfare Teaching Hospital, Medical City Complex, and the Ministry of Health, Iraq. Verbal consent was obtained from all participants. The patients were diagnosed by a pediatric oncologist consultant and the control group was screened for health status. All healthy children underwent a thorough medical evaluation, including medical history and standard laboratory tests, to ensure they had no underlying illnesses or conditions that could affect the study outcomes.

Exclusion criteria: Patients visiting the Children Welfare Teaching Hospital on follow-up, who have finished chemotherapy and other solid abdominal masses and Patients with chronic illnesses and non-cancerous conditions were excluded

Blood samples (5 mL) were collected from the peripheral veins of all participants and allowed to clot for 15 minutes. The samples were then centrifuged at 2500 rpm for 10 minutes, and the separated serum was stored at -45°C until laboratory analysis.

Metanephrine levels were measured using the enzyme-linked immunosorbent assay (ELISA) sandwich technique, employing the HumaReader system (Human Diagnostics, Germany). The assay utilized a biotin double antibody sandwich method to assess human Metanephrine. Wells were pre-coated with monoclonal anti-Metanephrine antibodies and incubated with the samples. Streptavidin-HRP was then added and allowed to form an immunological complex with biotin-labeled antibodies. Following incubation and washing, unbound enzymes were removed. Substrate A and B solutions were added, resulting in a color change from blue to yellow upon

acidification. The intensity of the color change was inversely proportional to the Metanephrine concentration.

Lactate Dehydrogenase levels were determined using the Roche Cobas analyzer, following the International Federation of Clinical Chemistry (IFCC) recommended method. In the presence of the cofactor NAD+, LDH catalyzes the conversion of L-lactate to pyruvate, simultaneously reducing NAD+ to NADH. Data analysis was performed using SPSS software (version 25.0; SPSS Inc., Chicago, IL, USA). Descriptive statistics, including mean and standard errors, were calculated for normally distributed data. The analysis of variance (ANOVA) test was employed to assess differences between the groups.

Results:

Table 1 shows the comparison between the mean values of age and anthropometric parameters at diagnosis among the pediatric tumor groups and controls. No significant differences were observed in age, or height across the groups.

Table 1: Age and anthropometric parameters at diagnosis among the study groups

Parameter	Neuroblastoma (n=34)	Wilms Tumor (n=31)	Control (n=33)	p-value
Age (months)	39.6 ± 4.84	40.0 ± 4.36	40.9 ± 5.58	0.980
Weight (kg)	15.0 ± 1.00	15.5 ± 0.91	15.4 ± 0.97	0.942
Length (cm)	92.0 ± 3.34	91.6 ± 2.88	92.9 ± 3.00	0.960

One-way ANOVA showed no significant differences between groups for age, weight, or length ($p > 0.05$).

Regarding Metanephrine serum levels among the study groups, the mean Metanephrine concentration in Neuroblastoma patients (26.2 ± 2.24 ng/mL) was markedly higher compared to both Wilms tumor patients (8.0 ± 0.28 ng/mL) and healthy controls (7.5± 0.29 ng/mL). There was more than a threefold increase in Metanephrine levels in Neuroblastoma patients relative to the other groups, which was statistically significant between Neuroblastoma patients and both Wilms tumor patients and controls (p-value < 0.001). In contrast, the comparison between Wilms tumor patients and healthy controls revealed no statistically significant difference in Metanephrine levels ($p = 0.956$).

LDH levels were significantly elevated in both tumor groups - Neuroblastoma (848.8 ± 87.57 U/L) and Wilms tumor (629.3 ± 66.99 U/L) - compared to the control group (68.6 ± 3.90 U/L; ANOVA, $p < 0.0001$) as shown in Table 2. Post-hoc analysis indicated significantly higher LDH levels in Neuroblastoma patients compared to Wilms tumor patients ($p < 0.0001$). Higher LDH levels in malignancies are often associated with tissue damage, hypoxia, and metastatic activity, potentially explaining the observed differences

Table 2: Mean serum levels of biochemical parameters across the three study groups

Metabolite	Control	Neuroblastoma	Wilms tumor	P value
Metanephrine ng/ml	7.5± 0.29	26.2^{a} ± 2.24	8.0^{b} ± 0.28	<0.0001 #
LDH U/L	68.6 ± 3.90	848.8^{a} ± 87.57	629.3^{b} ± 66.99	<0.0001 #

Mean ± Standard errors, # ANOVA, § T-test, a, b= groups with the same letter differ significantly in the post-hoc test according to Tukey-Kramer

Discussion:

The results of the current study showed that the mean metanephrine level in neuroblastoma patients was more than threefold higher than in Wilms tumour patients and healthy controls, which suggests that metanephrine may play a role in neuroblastoma pathogenesis or can be a byproduct of tumor activity. The highly significant differences between these levels indicate that this is unlikely to be due to chance, and support MN's potential as a specific NB biomarker. this finding is in agreement with Peitzsch (16). The absence of a significant difference between WT and controls regarding the same parameter suggests that MN elevation is not a feature of WT, which is in agreement with De Carvalho (17), reinforcing its specificity for NB. The marked elevation of MN in NB patients, coupled with its normal levels in WT and controls, suggests that MN could be a valuable diagnostic marker to differentiate NB from other pediatric tumors or non-malignant conditions. Since MN is a catecholamine metabolite, its increase in NB may reflect the tumor's neuroendocrine origin, similar to how pheochromocytomas or paragangliomas secrete catecholamines. This aligns with NB's derivation from neural crest cells. MN could aid in the early detection or monitoring of NB, especially in high-risk populations. MN levels may help distinguish NB from WT or other abdominal masses in children. Future study could explore whether MN levels correlate with tumor burden, stage, or response to therapy.

The results of the current study show that lactate dehydrogenase (LDH) levels were significantly higher in both NB and WT cases compared to controls. Post-hoc analysis indicated NB had markedly elevated LDH compared to WT, suggesting greater tumor burden or aggressiveness in NB. Elevated LDH in these

malignancies may reflect tissue damage, hypoxia, or metastatic activity, potentially explaining the observed differences. In an Austrian study, WT and NB cells exhibit increased glycolysis even under aerobic conditions, a phenomenon known as the Warburg effect. This metabolic shift requires elevated LDH activity to convert pyruvate to lactate, regenerating NAD^+ for sustained glycolysis. The hypoxia-inducible factor-1α (HIF-1α), often upregulated in solid tumors, further promotes glycolysis and LDH expression under hypoxic conditions. The current study agrees with Aminzadeh et al (18).

Elevated LDH in WT and NB results from hypoxia-driven glycolysis, oncogenic metabolic reprogramming, tumor necrosis, and metastatic burden. It serves as a prognostic marker, reflecting aggressive biology and advanced disease, the current study agreement with Dorneburg et al (19) and Kunc (20).

Limitations:

The study's sample size may limit the detection of smaller effect sizes. Future studies with larger cohorts are recommended to explore subtle or less pronounced effects. Additionally, ensuring that groups are matched for age, gender, and relevant risk factors will enhance the robustness of findings.

Conclusions:

The study emphasizes the importance of using metanephrine measurements for Neuroblastoma diagnosis and monitoring, as these metabolites can inform treatment response and disease progression. Neuroblastoma exhibits higher levels of metanephrine and lactate dehydrogenase, indicating a larger tumor burden. Wilms tumor does not share these characteristics**.**

Authors' declaration:

We confirm that all the Figures and Tables in the manuscript belong to the current study. Besides, the Figures and images, which do not belong to the current study, have been given permission for re-publication attached to the manuscript. Authors sign on ethical considerations' Approval-Ethical Clearance: The project was approved by the local ethical committee of (Department of Chemistry, College of Medicine, University of Baghdad) according to the code number (21) on (13/04/ 2025).

Conflict of Interest: None

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Data availability:Upon reasonable request, the corresponding author will make the data sets generated and/or analyzed during the current work available.

Author contributions:

Study conception & design: (Manal Kamal Rasheed&Hasanein Habeeb Ghali). Literature search: (Nihad Atea Jwda). Data acquisition: (Nihad Atea Jwda). Data analysis & interpretation: (Manal Kamal Rasheed, Hasanein Habeeb Ghali, Nihad Atea Jwda), Manuscript preparation: (Manal Kamal Rasheed, Hasanein Habeeb Ghali). Manuscript editing & review: (Manal Kamal Rasheed, Hasanein Habeeb Ghali).

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تقييم مستويات الميتانيفرين ونازعة هيدروجين اللاكتات في أورام ويلمز والورم الأرومي العصبي

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الخلاصة

الخلفية: ورم ويلمز والورم الأرومي العصبي هما من الأورام الخبيثة الشائعة لدى الأطفال. تمت دراسة العلامات البيوكيميائية مثل الميتانفرينات في البلازما ونازعة هيدروجين اللاكتات (LDH) في المصل لقيمها التشخيصية والتنبؤية. الميتانفرين، وهو أحد نواتج استقلاب الكاتيكولامينات، يرتفع بشكل خاص في الورم الأرومي العصبي بسبب إفراز الورم للكاتيكولامينات. قد ترتبط مستويات LDH المرتفعة، على الرغم من أنها غير محددة، بعبء الورم في كل من ورم ويلمز والورم الأرومي العصبي، مما يعمل كعلامة غير مباشرة لنشاط المرض.

الأهداف: لتقييم الميتانفرين في البلازما ونازعة هيدروجين اللاكتات كمؤشرات حيوية في الورم الأرومي العصبي وورم ويلمز لدى مرضى الأطفال.

المنهجية: أجريت الدراسة بين فبراير وديسمبر 2024 في مستشفى تعليمي حماية الطفل في المدينة الطبية ببغداد، العراق، وقسم الكيمياء الحيوية/ كلية الطب/ جامعة بغداد، على 98 طفلا دون سن 10 عاما، تم تقسيمهم إلى: المجموعة الأولى: 34 مريضا بورم الأرومة العصبية، المجموعة الثانية: 31 مريضا بورم ويلمز، والمجموعة الثالثة: 33 طفلا سليما يعملون كمجموعة تحكم. تم جمع عينات دم (5 مل) من جميع المشاركين. تم قياس مستويات الميتانفرين في البلازما باستخدام طريقةELISA، بينما تم تحديد مستويات نازعة هيدروجين اللاكتات باستخدام محلل Roche Cobas كما تم قياس المعايير الأنثروبومترية.

النتائج: لم تجد الدراسة فروقا ذات دلالة إحصائية في العمر أو الوزن أو الطول بين مجموعات الورم الأرومي العصبي، وورم ويلمز، ومجموعة التحكم . ومع ذلك، أظهر مرضى الورم الأرومي العصبي مستويات ميتانفرين أعلى بكثير (26.2 ± 2.24 نانوجرام/مل) مقارنة بورم ويلمز (8.0 ± 0.28 نانوجرام/مل) والمجموعات الضابطة (7.5 ± 0.29 نانوجرام/مل). وبالمثل، كانت مستويات LDH مرتفعة في الورم الأرومي العصبي (848.8 ± 87.57 وحدة/لتر) وورم ويلمز (629.3 ± 66.99 وحدة/لتر) مقارنة بالضوابط (68.6 ± 3.90 وحدة/لتر) حيث أظهر الورم الأرومي العصبي LDH أعلى من ورم ويلمز. تسلط هذه النتائج الضوء على الميتانفرين و LDH كمؤشرات حيوية رئيسية للتمييز بين الورم الأرومي العصبي والمجموعات الأخرى.

الاستنتاجات: تؤكد الدراسة على أهمية استخدام قياسات الميتافرين لتشخيص ومراقبة الورم الأرومي العصبي، حيث يمكن لهذه المستقلبات أن تقدم معلومات حول الاستجابة للعلاج وتطور المرض. يظهر الورم الأرومي العصبي مستويات أعلى من الميتانفرين ونازعة هيدروجين اللاكتات، مما يشير إلى عبء ورمي أكبر. لا يشارك ورم ويلمز هذه الخصائص.

الكلمات المفتاحية: الورم الأرومي العصبي، ورم ويلمز، نازعة هيدروجين اللاكتات، الميتافرين، سرطان الأطفال.