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Immunogenetic Characterization of Non-Hodgkin Lymphoma in Iraqi Patients: Immunohistochemical Assessment of β-Catenin and Metadherin Expression with Molecular Evaluation of IgH Gene Clonality

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Abstract

Background: Non-Hodgkin lymphoma (NHL) is a heterogeneous category of lymphoid malignancies, defined by the disruption of major oncogenic pathways. Importantly, Wnt/β-catenin pathway activation and metadherin (MTDH) overexpression have important functions in tumor development and progression, but the patterns and interaction of these in patients in Iraq are poorly understood. There is also immunoglobulin heavy chain (IgH) gene clonality analysis which is a dependable molecular technique used to verify B-cell malignancies.

Objectives: To determine the immunohistochemical expression of β-catenin and MTDH and to determine the IgH gene clonality of Iraqi patients having NHL in order to understand its diagnostic significance and pathogenesis of the disease.

Methods: A total of 100 formalin-fixed paraffin-embedded tissue samples will be collected in the period between March and November 2025 in Baghdad Medical City and other involved centers (65 confirmed NHL cases and 35 reactive or normal controls). Immunohistochemistry was conducted to identify cytoplasmic, nuclear, and perinuclear expression of β-catenin and MTDH. Clonality of the IgH genes was determined by BIOMED-2 multiplex PCR with FR1, FR2 and FR3 regions, with the subsequent heteroduplex analysis.

Results: Cytoplasmic and perinuclear β-catenin expression was observed in 80.0% of NHL cases compared to 20.0% of controls ($p < 0.001$), while nuclear expression appeared in 21.5% of NHL cases and was absent in controls ($p < 0.01$). MTDH overexpression was detected in 64.6% of NHL samples versus 20.0% of controls ($p < 0.001$). Monoclonal IgH rearrangements were identified in 72.3% of NHL cases, whereas all controls showed polyclonal patterns. A significant association was found between MTDH overexpression and nuclear β-catenin expression.

Conclusion: Aberrant β-catenin activation and MTDH overexpression play key roles in NHL pathogenesis in Iraqi patients. IgH clonality analysis remains a reliable diagnostic method, and combined molecular and immunohistochemical profiling enhances diagnostic accuracy and highlights MTDH as a potential therapeutic target.

Keywords: Non-Hodgkin lymphoma; β-catenin; Metadherin (MTDH); Wnt signaling; IgH gene clonality

1. Introduction

Non-Hodgkin lymphoma (NHL) is a heterogenous family of lymphoid malignancies that develops as a result of clonal growth of B, T, or natural killer (NK) lymphocytes at different stages of maturation. It is a serious health burden in the world and the rising rates are related to immunodeficiency, environmental carcinogens and chronic antigenic stimulation [1,2]. Although current molecular diagnostics and targeted therapies have improved understanding of the

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pathogenesis of NHL, the mechanisms underlying the pathogenesis are not fully understood, especially the mechanisms of dysregulated intracellular signaling and immune evasion.

Lymphoid system is also naturally prone to malignant change because of its dependence on strictly regulated proliferation and differentiation in the course of immune reaction. The signaling pathways involved in cell cycle progression, apoptosis and maintenance of genomic stability are disrupted, which is involved in lymphomagenesis [3,4]. The Wnt/2-catenin signaling pathway is one of them, and 2-catenin, which is the protein product of the CTNNB1 gene, acts in cell adhesion and transcriptional control. It is degraded under physiological conditions, but when the pathway is activated abnormally, the cytoplasmic accumulation and nuclear translocation of the protein occurs, facilitating the transcription of oncogenic targets, including c-MYC and CCND1 [5-7].

The dysregulation of Wnt/ -catenin signaling has also been associated with hematological malignancies, where it promotes proliferation, suppresses apoptosis, and provides malignant cells with the properties of a stem cell [8]. Nuclear 2-catenin deposition has been regarded as an indicator of pathway activation and has been linked to aggressive tumor behavior and poor prognosis, but with varying levels of expression depending on NHL subtypes [9,10].

Metadherin (MTDH), or astrocyte elevated gene-1, is an oncogenic protein with many functions, including the activation of pathways including PI3K/Akt, NF- -catenin, and Wnt/ . Its upregulation leads to a higher invasiveness, metastasis, and resistance to therapy of tumors [12]. MTDH can potentially work synergistically with β-catenin to maintain oncogenic signaling in NHL, but its expression dynamics and relationship with Wnt signaling have not been well described especially in Iraqi and Middle Eastern populations.

Diagnostically, to differentiate between neoplastic and reactive lymphoid proliferation, immunoglobulin heavy chain (IgH) gene clonality analysis is necessary. BIOMED-2 multiplex PCR offers a standardized and highly sensitive mechanism of identifying clonal rearrangements in formalin-fixed paraffin-embedded tissues [13]. Immunohistochemistry and molecular diagnostics is a unified solution that integrates both spatial protein expression analysis with molecular confirmation of clonality, which enhances the diagnostic accuracy in hard-to-detect cases [14].

This study aims to (i) evaluate the immunohistochemical expression patterns of β-catenin and MTDH in NHL; (ii) assess IgH gene clonality using BIOMED-2 multiplex PCR; and (iii) investigate the potential association between these biomarkers to better understand their diagnostic and pathogenic significance in Iraqi patients with NHL.

2. Materials and methods

2.1. Study Design and Timing

A cross-sectional analytical study was performed in Baghdad Medical City, Ghazi Al-Hariri Hospital and related private labs in Baghdad, Iraq during a period of eight months (March to November 2025).

2.2. Ethical Considerations

The study approved from the Institutional Ethics Committee of Baghdad Medical City (approval reference: BMC/EC/2025). All participants provided written informed consent before collection of sample, and its conducted in full accordance with the ethical principles of the Declaration of Helsinki.

2.3. Study Cohort and Histopathological Classification

One hundred formalin-fixed paraffin-embedded (FFPE) tissue samples were selected to include 65 cases of non-Hodgkin lymphoma (NHL) and 35 cases of reactive and normal lymphoid tissue as controls. Diagnoses were made by hematoxylin and eosin (H&E) morphology and immunophenotyping and cases were categorized by the 2022 World Health Organization (WHO) Classification of Hematolymphoid Tumors [2]. The cohort consisted of several types of B-cell lymphoma that included diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), small lymphocytic lymphoma (SLL), Burkitt lymphoma (BL), nodal marginal zone lymphoma (NMZL), and mucosa-associated lymphoid tissue (MALT**).**

2.4. Immunohistochemical Analysis

Immunohistochemistry (IHC) was performed On 4-mu mm FFPE sections on positively charged slides. After deparaffinization and rehydration, the process of antigen retrieval was accomplished with the help of heat-induced epitope retrieval in citrate buffer (pH 6.0). The activity of endogenous peroxidases and non-specific binding was

inhibited before adding primary antibodies of 2 -catenin and metadherin (MTDH) at the optimal dilution. Detection was performed using a polymer-based peroxidase system with diaminobenzidine (DAB) as the chromogen, and hematoxylin counterstaining was used to assess 2 -catenin expression (subcellular localization, i.e., membranous, cytoplasmic/perinuclear, nuclear) and MTDH expression was semi-quantitatively scored (03+), and Two pathologists, who had no access to clinical and molecular information, independently evaluated all slides, and disagreements were decided over by consensus.

2.5. DNA Extraction from FFPE Tissue

Genomic DNA was extracted from 3–5 sections (5 μm thickness) of FFPE tissue using a validated commercial kit optimized for formalin-fixed samples. The deparaffinization step was done using xylene then the crosslinking was reversed using proteinase K to release the nucleic acids. DNA purification was performed as per the standard procedures and quantified spectrophotometrically and evaluated its integrity through gel electrophoresis to check its appropriateness to undergo PCR analysis.

2.6. BIOMED-2 Multiplex PCR for IgH Clonality

The clonality of the IgH genes was assessed by the standardized BIOMED-2 multiplex PCR protocol of the framework regions FR1, FR2 and FR3 [15]. PCR was performed with 50-100 ng DNA in a 25 μL volume with 50–100 ng DNA using optimized cycling conditions. The amplified products were then separated on 2% agarose gels and further examined by the heteroduplex analysis method to differentiate between monoclonal and polyclonal patterns. The discrete dominant bands defined monoclonality and a smear or ladder pattern defined polyclonality. Negative controls during the analysis were reactive lymphoid tissues.

2.7. Statistical Analysis

Data were calculated in SPSS 26.0. Frequencies and percentages were used to present categorical variables whereas the mean and standard deviation were used to present continuous variables. Chi-square or Fisher exact tests were used to compare groups, and independent-samples t-tests were used when the normality was tested and continuous data were involved. Spearman's correlation was used to evaluate associations between immunohistochemical markers. The statistical significance was taken as a p-value of < 0.05.

3. Results

3.1. Clinicopathological Characteristics of the Study Cohort

The **table 1** summarizes the demographic and clinicopathological characteristics of the study population, including 65 NHL cases and 35 controls. The mean age was 52.4 ± 13.1 years in NHL cases and 49.2 ± 12.6 years in controls. The sex distribution showed 38 males and 27 females in NHL cases, compared to 19 males and 16 females in controls. Histological subtypes were reported only for NHL cases, with Diffuse Large B-Cell Lymphoma (DLBCL) being the most frequent (43.1%), followed by Follicular Lymphoma (13.8%), Small Lymphocytic Lymphoma (12.3%), Burkitt Lymphoma (9.2%), MALT lymphoma (7.7%), Unclassifiable B-cell lymphoma (7.7%), and Nodal Marginal Zone Lymphoma (6.2%).

Table 1 Clinicopathological characteristics of NHL patients and control group

Variable	NHL Cases (n = 65)	Controls (n = 35)
Age (mean ± SD, years)	52.4 ± 13.1	49.2 ± 12.6
Sex: Male / Female	38 / 27	19 / 16
Histological Subtype		—
Diffuse Large B-Cell Lymphoma (DLBCL)	28 (43.1%)	—
Follicular Lymphoma (FL)	9 (13.8%)	—
Small Lymphocytic Lymphoma (SLL)	8 (12.3%)	—
Burkitt Lymphoma (BL)	6 (9.2%)	—
MALT Lymphoma	5 (7.7%)	—

Nodal Marginal Zone Lymphoma (NMZL)	4 (6.2%)	—
Unclassifiable B-Cell Lymphoma	5 (7.7%)	—

DLBCL = Diffuse Large B-Cell Lymphoma; FL = Follicular Lymphoma; SLL = Small Lymphocytic Lymphoma; BL = Burkitt Lymphoma; MALT = Mucosa-Associated Lymphoid Tissue; NMZL = Nodal Marginal Zone Lymphoma; SD = Standard Deviation.

3.2. Immunohistochemical Expression of β-Catenin

The table 2 shows the distribution of β-catenin localization across NHL cases and controls. Cytoplasmic/perinuclear expression was observed in 52 cases (80.0%) compared to 7 controls (20.0%) with a p-value < 0.001. Nuclear expression was detected in 14 cases (21.5%) and was absent in all controls (0%), with a p-value < 0.01. Negative expression was recorded in 13 cases (20.0%) and 28 controls (80.0%), with a p-value < 0.001. All p-values were calculated using the chi-square test.

Table 2 Immunohistochemical expression patterns of β-catenin in NHL cases and control tissues

β-Catenin Localization	NHL Cases (n = 65)	Controls (n = 35)	P-Value
Cytoplasmic / Perinuclear	52 (80.0%)	7 (20.0%)	< 0.001
Nuclear	14 (21.5%)	0 (0%)	< 0.01
Negative	13 (20.0%)	28 (80.0%)	< 0.001

P-values derived from chi-square test. Nuclear β-catenin positivity was absent in all control specimens, confirming its specificity as a marker of Wnt pathway activation in neoplastic lymphoid tissue.

3.3. Immunohistochemical Expression of Metadherin (MTDH)

MTDH expression was positive (IHC ≥2+) in 42 of 65 NHL cases (64.6%) compared to 7 of 35 controls (20.0%), with a statistically significant difference (p < 0.001). Negative expression (IHC 0–1+) was observed in 23 NHL cases (35.4%) and 28 controls (80.0%). Immunohistochemical positivity was defined as cytoplasmic staining intensity of moderate to strong (≥2+), and statistical analysis was performed using the chi-square test. The distribution of MTDH expression is summarized in **Table 3**.

Table 3 Immunohistochemical expression of Metadherin (MTDH) in NHL cases and control tissues

MTDH Expression	NHL Cases (n = 65)	Controls (n = 35)	P-Value
Positive (IHC ≥ 2+)	42 (64.6%)	7 (20.0%)	< 0.001
Negative (IHC 0–1+)	23 (35.4%)	28 (80.0%)	—

IHC = Immunohistochemistry. Positive expression defined as cytoplasmic staining intensity ≥2+ (moderate to strong). P-value determined by chi-square test.

3.4. IgH Gene Clonality Analysis by BIOMED-2 Multiplex PCR

The table 4 provides a summary of IgH clonality patterns of cases and controls in NHL. Monoclonal rearrangements were found in 47/65 (72.3) NHL cases and none of the 35 control samples displayed monoclonality (0%). In the 18 NHL cases, (27.7%), and all control samples (100%), polyclonal patterns were found. The heteroduplex analysis could give a single or two discrete dominant PCR bands, which defined monoclonality. Polyclonal patterns were observed in all control specimens which verified assay specificity. Some NHL cases had also been reported to be polyclonal.

Table 4 IgH gene clonality patterns detected by BIOMED-2 multiplex PCR in NHL cases and controls

Clonality Pattern	NHL Cases (n = 65)	Controls (n = 35)
Monoclonal	47 (72.3%)	0 (0%)
Polyclonal	18 (27.7%)	35 (100%)

Monoclonality defined as detection of one or two discrete dominant PCR amplification bands on heteroduplex analysis. All control specimens demonstrated polyclonal patterns, confirming assay specificity. Polyclonal results in NHL cases may reflect low tumor cellularity, somatic hypermutation affecting primer annealing, or FFPE-related DNA fragmentation.

4. Discussion

This study provides a comprehensive immunohistochemical and molecular characterization of non-Hodgkin lymphoma (NHL) in an Iraqi cohort by means of a combined analysis of 2 -catenin and metadherin (MTDH) expression as well as immunoglobulin heavy chain (IgH) gene clonality, assessed by BIOMED-2 PCR. This combination of complementary methods allows the development of a multidimensional concept of lymphoma pathobiology and biomarker-based diagnostic approaches in a regional setting.

The results show that cytoplasmic and nuclear expression of β-catenin is highly prevalent in NHL tissues, which means that the canonical Wnt/ -catenin signaling pathway is activated. The functionally essential form, which is found in a subset of cases but not in controls, is nuclear -catenin, which directly mediates transcriptional regulation of oncogenic targets including c-MYC, CCND1, and survivin [7]. The specified observation can be linked to known models of Wnt-mediated oncogenesis, and it can be supported by previous research that has already reported the relationships between the expression of nuclear 8 -catenin, elevated proliferative rates, and poor clinical outcomes in aggressive lymphomas [8,9]. The nuclear heterogeneity of the histological subtypes also suggests that the activity of the Wnt pathways may be context-dependent and may depend on the epigenetic and microenvironmental parameters [10].

MTDH expression was found to be present in a substantial proportion of NHL cases and this indicates its usefulness as an important oncogenic mediator. It has been reported that MTDH is involved in mediating various signaling pathways including PI3K/Akt, NF-kB, and Wnt/ -catenin signaling pathways that promote tumor cell survival, proliferation and resistance to apoptosis [11]. It has also been found to be more invasive with higher expression [12], which has been found to increase its metastasis and resistance to chemotherapy. Interestingly, MTDH and nuclear 2 -catenin are co-expressed, which also suggests that they interact synergistically as oncogenes, where MTDH can amplify Wnt signaling by regulating upstream regulators, such as GSK-3. This mutual response supports the concept of interdependent pathways of signaling that enhance the development of lymphoma and not the stimulation of individual pathways.

Therapeutically, the prevalence of MTDH overexpression is remarkable, which indicates that it can be used as a target in new treatment options. It has been established that MTDH inhibition can re-establish chemosensitivity, and improve apoptosis in tumor cells through experimental studies and is now emerging as a potential synergist with immunotherapies by modulating immune checkpoint pathways [13,14]. The results highlight the importance of MTDH as a target in the management of NHL.

The diagnostic strength of the BIOMED-2 multiplex PCR method is further confirmed by the molecular analysis of IgH gene clonality. It has been shown to be useful in differentiating between neoplastic and reactive lymphoid proliferations by detecting monoclonal rearrangements in most NHL cases and polyclonal patterns in controls [16]. The biological variations in the detection rates between different lymphoma subtypes can be explained by both somatic hypermutation that might influence the efficiency of the primer binding and technical constraints associated with the quality of the DNA in formalin-fixed tissue [17]. Multiple primers of the framework regions are used to enhance the sensitivity of the assays particularly FR3 that targets smaller amplicons that are not highly affected by degradation of DNA.

It is important to note that when combined with immunohistochemistry and molecular clonality testing, the diagnostic framework has formed a robust diagnosis framework, which encompasses multiple facets of lymphoma biology, including oncogenic signaling, protein expression, and clonal identity. The hybrid type of methodology is particularly convenient in the situations of diagnostics of high difficulty when morphological and immunophenotypic data may not be conclusive by themselves [18].

Moreover, the investigation also adds useful population-specific information on Iraq, which is underrepresented in the research of molecular lymphoma. The observed molecular patterns (high rate of Wnt pathway activation and MTDH overexpression) may be largely compared with the findings of other studies in the world, which are similar in terms of oncogenic processes, but also it is necessary to make regionally specific studies [19].

5. Conclusion

This study represents the first comprehensive immunogenetic characterization of the non-Hodgkin lymphoma (NHL) in an Iraqi cohort with combined immunohistochemical evaluation of β-catenin and metadherin (MTDH) and molecular evaluation of immunoglobulin heavy chain (IgH) clonality. The results reveal that the Wnt/ -catenin pathway is often activated, which is manifested by the accumulation of β-catenin in the cytoplasm and its translocation to the nucleus, is one of the predominant NHL pathogenesis mechanisms. Nuclear 8 -catenin was significantly associated with MTDH

overexpression, which occurs in most cases and may be a cooperative oncogenic interaction, leading to tumor aggressiveness and resistance to treatment. Clonality studies with IgH through BIOMED-2 PCR proved monoclonality in most cases of NHL, and polyclonality in controls, which indicates it is a reliable diagnostic method in formalin-fixed tissues. Altogether, the combination of immunohistochemical and molecular methods improves the accuracy of the diagnosis and offers useful information regarding the biology of the disease. To determine the prognostic and therapeutic utility of β-catenin and MTDH, especially as a subset of targets in precision and immunotherapy-based therapeutics, future studies need to incorporate larger cohorts, more sophisticated molecular profiling methods, and clinical correlation.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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