Study the expression of β – catenin and MTDH in Iraqi patients with Non-Hodgkin’s lymphoma

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Abstract
Non-Hodgkin’s lymphoma (NHL) is a cancer that originates in our lymphatic system. β-catenin is a protein encoded by the catenin β (CTNNB1) gene, it has two functions as gene transcription and regulation of cell-cell adhesion. Metadherin (MTDH) is associated with tumor cell progression, such as cell proliferation, invasion and metastasis by activating abnormally various oncogenic signalling pathways such as, nuclear factor kappa B (NF-κB), phosphatidylinositol 3-kinase (PI3K)/Akt and wingless (Wnt)/ β-catenin pathways. In this study 56 formalin fixed paraffin embedded tissue (FFPE) were collected and the detection for β –catenin and (MTDH) was carried out by using immunohistochemical analysis (IHC). The results of IHC were revealed positive staining in cytoplasmic as a perinuclear of β -catenin in tumour cell that was detected in 78% (29/3) case of non-Hodgkin’s lymphoma and 20% (7/37) that was negative for b-catenin, while 21% (4/19) case of normal lymphoid tissue were showing a positive staining outside the germinal centre and in all case of normal lymphoid tissue showed the positive staining in germinal centre. Nuclear β –catenin was observed in 18% (7/37) cases of non-Hodgkin’s lymphoma, while no positive staining in nuclear was shown in normal lymphoid tissue. MTDH was detected as a positive in 64% (24/37) cases non-Hodgkin’s lymphoma, while 36% (13/37) cases were detected as a negative for MTDH. In normal lymphoid tissue, 79% (15/19) of cases also detected as a negative for MTDH and 21% (4/19) of case was showed positive staining for MTDH in lymphocytes. Based on the statistical analysis, there was a significant difference between nuclear β-catenin protein expression in non-Hodgkin's patients and control samples despite the small percentage shown by the results. MTDH expression in NHL cells can be enhanced cell proliferation, which leads to inhibition the apoptosis of cells. MTDH was overexpressed in NHL with nuclear Wnt/β-catenin expression, and confirmed that MTDH worked as an oncogene to promote lymphoma cells proliferation and inhibit their apoptosis through wingless-type (Wnt) β-catenin signal pathway

Keyword : Non-hodgkin lymphoma, β-catenin, IHC, MTDH

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Introduction
Non Hodgkin lymphoma is a cancer that originates in our lymphatic system, the disease-fighting network spread throughout the body. In non-Hodgkin’s lymphoma, tumours develop from lymphocyte – type of white blood cell. The B cell lymphomas are “blood cancers “in lymph nodes. They develop more frequently in older adults and in immunocompromised individuals”[1]. Non-Hodgkin lymphomas (NHLs) are a heterogeneous group of neoplastic disorders in which malignant lymphocytes have arrested at various stages of differentiation and have acquired the ability to proliferate, evade the host’s immune system, and avoid programmed cell death (cellular apoptosis)[2]. The malignant cell for many lymphomas can be traced to a specific stage in lymphoid maturation, with the majority derived from mature B cells or those of germinal centre origin. There are many different types of non-Hodgkin lymphoma (NHL), about 90% of B cell lymphoma, 9% of T cell lymphoma and less than 1% of Natural killer cell (NK) lymphoma, B and T lymphoma also divided into several subtype[3]. Non-Hodgkin lymphoma (NHL) is one of the most common cancers in the United States in 2017, accounting for about 4% of all cancers and 6th most common cancer in the UK, accounting for 4% of all new cancer cases in (2015) [4][5].
In Iraq NHL was the fourth of the commonest ten cancers in 1995-1999 after breast, lung and urinary bladder, while in 2002 it was the fifth after breast, lung, urinary bladder and brain and CNS, in 2003-2013 NHL it was the fourth of the commonest ten cancers in Iraq after breast, lung, and leukaemia[6]. Many factors found that effects to persons to get non-hodgkins lymphoma such as age, gender, race, ethnicity, and geography, exposure to certain chemicals and drugs, radiation exposure ,having a weakened immune system autoimmune diseases, certain infections, body weight and diet, Breast implants, infection with virus and bacteria but the cause of most lymphomas is not known [7]. NHL are neoplasms that arise from lymphoid cells of either B-cell or T-cell lineage [8].

In humans, β-catenin protein encoded by the CTNNB1 gene and β-catenin has two functions, firstly gene transcription and coordination and second regulation of cell-cell adhesion[9]. In adults, Wnt/β-catenin signalling pathway plays an important role in maintenance of organs and tissues as well as embryonic development[10]. From the point of view, β-catenin binds to the cytoplasmic portion of E-cadherin, on the other hand, it can translocate to the nucleus and activate cell proliferation. B-Catenin is an important component of the so-called Wnt signalling pathway that regulates cell proliferation [11]. In leukaemia, myeloma the Wnt/β-catenin pathway has an important role in the increasing of aggressiveness of these diseases and many subtypes of NHL, that includes Burkett’s lymphoma (BL), cutaneous lymphoma extra nodal marginal zone lymphoma, Epstein-Barr virus (EBV)-positive, mantle cell lymphoma (MCL), anaplastic large cell lymphoma, small B-cell lymphoma (SBCL), diffuse large B-cell lymphoma (DLBCL), marginal zone B-cell lymphoma (MZBL) and follicular B-cell lymphoma (FL) [12-14]. Metadherin, known as astrocyte elevated gene-1 protein (AEG-1), also known as protein lysine-rich) LYRIC[15]. In cancer the MTDH participates in its progression, metastasis and invasion [16]. In recent years it has been confirmed that MTDH play an important role in many types of human malignancies [17]. By activation of Wnt/b-catenin pathway MTDH also redound to the pathogenesis of DLBCL, MALT and SLL[18-20].

Materials and methods
Formalin Fixed Paraffin-embedded tissue (FFPE) block samples
The study included Formalin fixed paraffin-embedded tissue (FFPE) for patients with non-Hodgkin’s lymphoma (NHL) was collected from September 2017 to January 2018. These samples were collected from Baghdad Teaching Hospital and Ghazi Al-Hariri Hospital in medical city (Baghdad). Ethical permission to conduct the research was obtained from ministry of health; selection of the samples was accomplished with assistance of pathologists in the hospitals. The study carried out on 56 FFPE samples (37 for patients with non-Hodgkin's lymphoma and 19 for normal lymphoid tissue that were used as control group). Clinical information was collected seeking his/her hospital record as well as previous medical history. Samples were included 20 men and 17 women with an average age of 46 years and range of 20 to 72 years. Histopathological type was classified according to the World Health Organization (WHO) (2008) as 15 diffuse large B cell lymphoma (DLBCL), 4 small lymphatic lymphoma (SLL), 3 follicular lymphomas (FL), 3 Burkett’s lymphoma (BL), 2 Nodal marginal zone lymphoma (NMZL), 2 Mucosa-associated lymphoma tissue (MALT) and 8 case with an unclassifiable lymphoma, which was categorized as B cell lymphoma (BCL)

Immunohistochemical analysis
Immunohistochemical staining technique briefly, 4 μm-thick tissue sections were deparaffinised in xylene and hydrated by immersing in a series of graded ethanol. Antigen retrieval was performed by using high pH at 95°C in water bath for 40 min. Endogenous peroxidase was inhibited by added peroxide block was added and incubated for 10 min in humid chamber power block was added and incubated for 10 min in humid chamber. Power block was added and incubated for 10 min in humid chamber. The primary antibody was added and incubated for 20 min, the HRP (Horseradish peroxidase) secondary antibody was added and incubated for 10 min Mayer’s haematoxylin added for 1 min as counter stain, the slides mounted by cover slides and be ready to be examined and do scoring (DAKO, USA).

Statistical analysis was used to differences between two variables and the experimental subjects and the controls were analyzed by the chi-squared statistic with different degrees of freedom.
Results
Expression of β-Catenin in non-Hodgkin's lymphoma
The expression of β-Catenin protein in 56 case including 37 patients with NHL and 19 cases with normal lymphoid tissue was investigated by IHC staining. The β-catenin expression of cytoplasmic as perinuclear staining was considered positive when at least 10% of tumour cells was expressed [12] and at least 30% of nuclear staining was considered as a positive [14]. The results were summarized in (Table 1).

Table 1: Expression of β-catenin in NHL and normal lymphoid tissue.

<table>
<thead>
<tr>
<th>Type</th>
<th>Cytoplasmic as perinuclear / n</th>
<th>Nuclear / n</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLBCL</td>
<td>14/15 (93%)</td>
<td>6/15 (40%)</td>
</tr>
<tr>
<td>Other NHL type</td>
<td>15/22 (68%)</td>
<td>1/22 (4%)</td>
</tr>
<tr>
<td>Total</td>
<td>29/37 (78%)</td>
<td>7/37 (18%)</td>
</tr>
<tr>
<td>normal lymphoid tissue</td>
<td>4/19 (21%)</td>
<td>0/19 (0%)</td>
</tr>
</tbody>
</table>

The positive control showed in (Fig 1-A) and the negative control showed in (Fig 1-B). The positive staining in cytoplasmic as perinuclear of β-catenin in tumour cell was detected in 29/37 (78%) case of NHLs (Fig 2-A) and 8/37(21%) was negative for β-catenin (Fig 2-B). Fourteen out of 15 (93%) of DLBCL and 15/22 (68%) in other NHL type, no positive staining was shown in normal lymphoid tissue and positive staining was showed outside the germinal centre and in all case of normal lymphoid tissue showed the positive staining in germinal centre 4/19 (21%) (Fig 3-A and 3-B). Nuclear β-catenin was observed in 7/37 (18%) case of non-Hodgkin’s lymphoma that divided into 6/15 (40%) case of diffuse large B cell lymphoma (Fig 4-4-A) and 1/4 (25%) case of small lymphatic lymphoma (Fig 4-B) and no nuclear staining was showed in other type of NHL and not even in normal lymphoid tissue.

Figure 1: The IHC staining of β–catenin protein A- Positive control (nuclear and cytoplasmic staining) of β-catenin in colorectal cancer B- Negative control of β-catenin in non-Hodgkin lymphoma cells without antibody (400x).
Figure 2: A- Dot like (perinuclear) as cytoplasmic staining of β-catenin in non-Hodgkin lymphoma (1000x) B- Non-Hodgkin lymphoma cells showed negative staining for β-catenin (400x)

Figure 3: A- The negative staining of β-catenin in normal lymphoid tissue and the positive staining shown in germinal centre (GC) B- The negative staining of β-catenin in normal lymphoid tissue, (400x).

Figure 4: A- Positive nuclear staining of β-catenin in DLBCL. B- Positive nuclear staining of β-catenin in SLL (400x).

Expression of MTDH in non-Hodgkin’s lymphoma and normal lymphoid tissue

The positive control showed in (Fig 5-A) and the negative control showed in (Fig 5-B). In this study the levels of MTDH was observed in 24/37 (64%) case NHLs, while 13/37 (36%) negative for MTDH (Fig 6-A). The pattern of expression was divided into low (Fig 7-A) and high (Fig 7-B) expression according to [16]. In normal lymphoid tissue 15/19 (79%) of case negative for MTDH (Fig 6-B) and 4/19 (21%) of case was showed positive staining for MTDH in lymphocytes. The results were summarized in (Table 2)
Table 2: Expression of MTDH in NHL and normal lymphoid tissue.

<table>
<thead>
<tr>
<th>Type</th>
<th>Low expression / n</th>
<th>High expression / n</th>
<th>MTDH+/n</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLBCL</td>
<td>4/15 (26%)</td>
<td>5/15 (33%)</td>
<td>9/15 (60%)</td>
</tr>
<tr>
<td>Other NHL type</td>
<td>7/22 (31%)</td>
<td>8/22 (36%)</td>
<td>15/22 (68%)</td>
</tr>
<tr>
<td>Total</td>
<td>11/37 (29%)</td>
<td>13/37 (35%)</td>
<td>24/37 (64%)</td>
</tr>
<tr>
<td>Normal lymphoid</td>
<td>2/19 (10%)</td>
<td>2/19 (10%)</td>
<td>4/19 (20%)</td>
</tr>
</tbody>
</table>

The positive staining of MTDH in NHL included into 9/15 (60%) case of DLBCL and in other NHL type 15/22 (68%), the low expression was observed in 11/37 (29%) while the high expression was observed in 13/37 (35%). MTDH expression was detected mainly appears much membrane, less cytoplasm and occasionally in the nucleus (Fig 4-7-C). In normal lymphoid tissue divided into 10% for low and high respectively.

Figure 5: The IHC staining of MTDH protein A- Positive control (cytoplasmic staining of MTDH in stomach cancer) B- Negative control (non-Hodgkin lymphoma cells without antibody) for MTDH (400x)
Figure 6:A- Non hodgkin lymphoma cells showed a negative staining for MTDH B-Negative staining of MTDH in normal lymphoid tissue (400x)

Figure 7:A- Low expression of MTDH in membrane and cytoplasmic in non-hodgkin lymphoma B- High expression of MTDH in membrane and cytoplasmic in non-hodgkin lymphoma C- positive staining in cytoplasm and occasionally in the nucleus of MTDH in non-hodgkin lymphoma. (400x).
Discussion

**Comparison β-catenin expression in non-Hodgkin's lymphoma and normal lymphoid tissue:**

In adults, Wnt/β-catenin signalling pathway plays an important role in maintenance of organs and tissues and in embryonic development [10]. A number of main processes were regulated during development by Wnt/β-catenin signalling pathway, such as cell polarity, proliferation, survival, differentiation and angiogenesis [14]. At cell adhesion junctions β-catenin was localized in epithelial cells and, in many cases, it was collected in the nucleus and activated the Wnt/β-catenin pathway and leads to uncontrolled transcription of target genes (including c-jun, cyclin D1, c-myc and MMP-7) that regulating cell adhesion, cell proliferation, and survival [21]. Deregulation of β-catenin was implicated in the pathogenesis of a series of diseases, such as many types of carcinomas, many studies have confirmed that [22]. This study observed the cytoplasmic localization as prenuclear dot like staining in (78%) of NHL, the dot like staining was observed so may can suggests the possibility that the cellular localization of β-catenin is in the Golgi apparatus, such results were showed in several studies [12][13][23].

This is in contrast to the situation in normal lymph node tissues, in which no nuclear or little cytoplasmic or prenuclear dot like (21%) of case accentuation of β-catenin was found outside the germinal centre and this results agreed with [14][24]. The prenuclear dot like staining it was showed in normal lymphoid tissue in study of [13]. This study suggestion the appearance of prenuclear dot like staining of β-catenin may be due to the activation of the Wnt/β-catenin pathway because of that β-catenin is a transcriptional factor that travels to the nucleus [14]. WNT pathway, which is essential for early T-cell and B-cell development [25] and plays a role in the self-renewal of hematopoietic stem cells, is aberrantly activated in a substantial subgroup of NHLs and contributes to lymphomagenesis [24].

Accentuation of β-catenin in the cytoplasmic may be the tumour microenvironment contributes to increasing β-catenin amounts [26] thus, the increased β-catenin levels could be at least partially a result of autocrine or paracrine Wnt signals within the tumour microenvironment [23]. Another reason to accentuation of β-catenin in the cytoplasmic may related to point mutations in APC or CTNNB1 [24][27], or due to the loss of E-cadherin tumour cell that lead to separated b-catenin from E-cadherin and moved to the cytoplasmic [11]. The nuclear localization was observed in (40%) of DLBCL and this results was agreed with previous study [14] [24] and 25% of case in SLL was revealed the nuclear localization and this result was agreed with [13]. No nuclear confirmation of β-catenin was found as showed in (Table 3) in contrast to the situation in normal lymph node tissues, in which and we founded significant difference (p >0.05) between NHL and control.

**Table 3:** Nuclear β-catenin expression in NHL patient compared with control.

<table>
<thead>
<tr>
<th>β-catenin Type</th>
<th>negative</th>
<th>positive</th>
<th>Total</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NHL</td>
<td>30</td>
<td>7</td>
<td>37</td>
<td>0.043*</td>
</tr>
<tr>
<td>control</td>
<td>19</td>
<td>0</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
<td>7</td>
<td>56</td>
<td></td>
</tr>
</tbody>
</table>

P-value <0.05

β-catenin works like a transcriptional co activatorIn the nucleus for the TCF/LEF family of transcription factors [28], β-catenin-TCF/LEF signaling pathway. In the last stage, β-catenin binds to pontin 52-TATA-binding protein in nuclear and transfers to Groucho-related gene or CREB-binding protein co repressors from TCF/LEF that lead to activation important transcription growth regulatory genes, such as cyclin D1 and c-Myc [29]. The most nuclear localization in DLBCL type and it is also recognized that activation of the Wnt/β-catenin pathway is an important factor in the pathogenesis of DLBCL. In many cases β-catenin gathers in the nucleus and is the main reason for activating the Wntβ-catenin signaling pathway, in nuclear β-catenin pathway could be a use as therapeutic factor for DLBCLand SLL and may contribute in to the pathogenesis of DLBCL and SLL [14], the SLL type, were needed to examine a large number of cases with β-catenin to confirmation the role of b-catenin in SLL. The absence of β-catenin in the nucleus for other type of non-Hodgkin lymphoma and remaining 9 case of DLBCL correlates with no detectable expression of target genes for β-catenin/Tcf-induced transcription such as cyclin D1. The absence of nuclear distribution of b-catenin could be correlated with the loss of expression of lymphoid enhancer factor-1 (LEF-1) and T-cell factor-1 (TCF-1) in the...
neoplastic cells. TCF-1 and LEF-1 are expressed in thymocytes and mature T cells in mice and human, LEF-1 is also expressed in B-cell precursors, but not in mature B cells [30]. When b-catenin is absent, the transcription factor TCF-1/LEF-1 is inactive and bind with co repressors (TLE/Groucho and/or CtBP) of the target gene promoters [31].

Comparison of MTDH in non-Hodgkin’s lymphoma:
The NHL cells was express high MTDH. This study observed 24/37 (64%) expression of MTDH in NHL analysed by immunohistochemical staining, while in normal lymph node tissues little or non-expression of MTDH was observed. The statistical analysis showed significant difference (p < 0.05) between NHL and control (Table 5-6) similar to previous studies [18] [20] [32] as shown in (Table 4).

Table 4: The statistical analysis of MTDH expression in NHL patient compared with control.

<table>
<thead>
<tr>
<th>MTDH</th>
<th>Negative</th>
<th>Positive</th>
<th>Total</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type</td>
<td>Patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>control</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>13</td>
<td>24</td>
<td>37</td>
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<td></td>
<td></td>
<td>15</td>
<td>4</td>
<td>19</td>
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<tr>
<td></td>
<td></td>
<td>28</td>
<td>28</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.002*</td>
</tr>
</tbody>
</table>

P-value <0.05

Several studies have shown that MTDH was highly expressed in a host of human malignancies, such as breast cancer, lymphoma and urologic neoplasms so on [33]. Furthermore, neoplasm evolution, development, invasion/tumour progression, evolution and metastasis association with MTDH by activating abnormally various oncogenic signalling pathways as previous studies explained, such as, nuclear factor kappa B (NF-kB), phosphatidylinositol 3-kinase (PI3K)/Akt and wingless and IN T-1 (Wnt)/β-catenin pathways [34], in hepatocellular carcinoma cells it was hypothetical that MTDH carries β-catenin the vital element of Wnt/β-catenin pathway into nuclear translocation and activating the Raf/MEK/mitogen-activated protein kinase (MAPK) signal pathway and cause up regulates different target gene expressions [35]. MTDH is a novel oncogene in carcinogenesis was abnormal expression that associated with diverse biological behaviours in tumour progression, including modulating cell proliferation and apoptosis, invasion and migration and drug resistance [16][32].

In study [35] confirmed that MTDH was connected with angiogenesis in breast cancer. In last years, MTDH has been confirmed to have a role in human malignancies and its expression excessively higher in prostate cancers, malignant glioma cell lines, melanoma, neuroblastoma, gastric, hepatocellular and breast, renal cell carcinoma, and oesophageal compared with their normal samples [17]. In DLBCL type it has been observed 9/15 (60%) case positive for MTDH and this result agreed with previous studies [18] [32]. In Mucosa associated lymphoma tissue (MALT) type it has been observed 2/2(100%) case positive for MTDH and this result is agreed with study [20], and it was showed the positive staining for MTDH in FL 2/3 (66%), BL 2/2 (100%), NMZL 2/2(100%), SLL 3/4(75%) and 4/8(50%) in BCL. This study suggested that expression of MTDH in NHL cells could enhance cell reproduction, which leads to inhibition the apoptosis of cells according to study [18].

Conclusions
1- Since MTDH was overexpressed in patients with NHL, so it can be used as a good diagnostic tool and a good therapeutic target.
2- Based on the statistical analysis, there was a significant difference between nuclear β-catenin protein expression in non-Hodgkin’s patients and control samples despite the small percentage shown by the results

References


