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Incidence and outcome of histological transformation in a single-institution cohort of patients with follicular lymphoma

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Background: Few reports describe the histological transformation of follicular lymphoma into diffuse large B-cell lymphoma with respect to risk factors and subsequent clinical outcome.
Patients and Methods: The database of the Oncology Institute of Southern Switzerland (IOSI) contains information of 281 patients with FL treated from 1979 to 2007, including clinical features at diagnosis, therapeutic approaches, survival patterns, with special reference to the impact of histological transformation on disease course. Median survival times, Kaplan-Meier survival curves and relative survival rates were calculated.
Results: Median age at diagnosis was 58 years (range 21−92 years). Median follow-up time from diagnosis was 10 years in the entire cohort and the median overall survival was 11 years (95% CI, 8.8−14 years). Histological transformation into diffuse large B-cell lymphoma was observed in 39 patients (14%; 95% CI, 10−18%). The median time to transformation was 5 years from diagnosis. Risk of transformation at 5, 10 and 14 years was 13% (95% CI, 9−18%), 16% (95% CI, 12−22%), 27% (95% CI, 19−38%), respectively. Notably, histological transformation was not diagnosed in 30 patients (of whom 13 have died) with a minimum follow-up of 14 years (range 14−29; median, 17 years); the rate of transformation remained at 27% from that point onward. This seems to confirm a recent report that there is a subgroup of patients in whom histological transformation may not occur. The histological transformation was associated with a significantly shorter cause specific survival (CSS) (P = 0.0003), which in the patients who did not experience histological transformation was 74% at 10-years (95% CI, 66−80), as opposed to 44% (95% CI, 26−61) in those with transformation. The median survival after transformation was 3 years. The risk of histological transformation was higher in the group of patients diagnosed before 1989 compared to the subsequent period (P = 0.03). In our series an initial "watch and wait" policy appeared associated with a lower risk of subsequent transformation, in comparison with treatment being initiated at diagnosis (P < 0.05). No other therapeutic approach seems to impact the risk of histological transformation.
Conclusions: Our data confirmed the adverse clinical outcome of FL after histological transformation. Patients undergoing expectant management at diagnosis did not show a higher risk of transformation and the effect of specific therapeutic strategies on histological transformation needs to be further explored.

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DNA reparation genes in genetic and epigenetic susceptibility to Chronic Lymphocytic Leukaemia

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Chronic lymphocytic leukaemia (B-CLL) is one of the most common malignant lymphoid diseases in the western world. An early event in this pathology is the appearance of chromosomal deletions, insertions and translocations generated by Double Strand Breaks (DSB) incorrectly repaired. These repair failures could be due to the existence of genetic and/or epigenetic variations in the genes of this pathway. Consequently, we considered the identification of low penetrance susceptibility alleles and variations in methylation levels of the DSB repair genes, centring mainly on the Non-Homologous NHEJ pathway. We carried out a case-control study, genotyping 89 SNPs in eight genes in the NHEJ pathway (ATM, ATR, XRCC4, XRCC5, XRCC6, XRCC7, LIG 4 and DCLRE1C) in 691 cases and 728 controls. Genotyping was performed by using Illumina Bead Array System (Illumina Inc., San Diego, USA) and the MassARRAY SNP genotyping system (Sequenom Inc., San Diego, CA). Methylation status of the gene promoters in the NHEJ pathway was analyzed by MSP (methylation specific PCR) in 150 cases and 150 controls. In the single-locus analysis, we found a strong association with CLL risk after stringent adjustment for multiple testing in the ATM gene variant, rs228589, situated in 5′region. Allele T was significantly more frequent in cases than in controls (41% versus 36%; odds ratio 1.24; 95% confidence interval 1.065−1.439; P-permutation = 0.048). No significant association was found for 81 other polymorphisms studied. Moreover, we have found association with B-CLL risk for 2 haplotypes in ATM gene: one risk ATM haplotype "TCGTTCTTATCGT" (OR = 1.325; 95% CI, 1.102−1.594; Global P-permutation = 0.04 after permutation testing) and one protective "CCGATCTTGTCGG" (OR = 0.824; 95% CI, 0.707−0.962; Global P-permutation = 0.04). Methylation analysis showed 2 genes differentially methylated. CpG island of LIG4 gene promoter was significantly more methylated in CLL patients, while XRCC5 showed a higher methylation frequency in controls. These results, based on an extensive number of patients and controls, will provide new insights in the development of CLL.

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Clinical and morphological characteristics of primary non-Hodgkin's thyroid gland lymphoma

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Primary thyroid lymphoma is rare disease (2.5−7% of all extranodal lymphomas). The aim of our review were determination of clinical and hystopathologic characteristics of this type lymphoma.
Materials and Methods: 704 patients with primary extranodal non-Hodgkin's lymphomas were observed in CRC of RAMS sins 1983 to 2007. 39 patients (5.5%) were affected by primary lymphoma of thyroid gland. Diagnosis was determined after hemythyreoidectomy or open biopsy of the tumor with following immunohystochemical method investigation.
Results: Median of observation was 60 months. 29 patients (74%) female, 10 (26%) male. Age at time of diagnosis ranged from 15 to 83 years (the median age-51 years). 26 patients (66.7%) was younger 60 age. The association Hashimoto's disease and lymphoma has been found in 4 cases of female (10.2%). Most patients (78%) presented for painless neck edema. Bulky disease were expose in 1/2 of cases (21 patients). Predominance histological type was diffuse large B-cell lymphoma 14 (36%) cases. T-cell lymphoma 2 (5.1%) and Burkitt lymphoma 4 (10%), follicular 1 (2.5%), other B-cell lymphoma 11 (28.2%), MALT type and mantle cell lymphoma 1 (2.5%). In 5 cases histological type was not determined. IV stage of disease was constituted in 10 cases (25%). IE stage was determined in 11 cases (28%), IIE -in 13 (33%), IIIE 13% clinical cases. 14 patients (36%) had B-symptoms. In according to parameters of ECOG: 0−1 mark 48%, 2 23%, 3 28%, 4 were not observed. In according to parameters of International Prognostic Index 18 patients (46%) were attitudes to poor prognostic group and had high risk of progressive disease. 21 (54.2%) patients had favourable prognosis. LDG level exceeds normal index was in 29 cases. Level of Hb less 12 g/l was in 16 cases.
Conclusions: Predominantly lymphoma occurs in female population. 2/3 of patients were younger 60 age. Predominance histological type was diffuse larg B-cell lymphoma. In according to parameters of ECOG: 0−1 mark were 48% cases and 54.2% patients had favourable prognosis.

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Primary breast lymphoma is a rare presentation: patient profile and treatment outcome

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Background: Primary breast lymphoma (PBL) is a rare entity. Many reports showed that mastectomy offered no benefit in the treatment of PBL. However, there is no well-defined treatment strategy.
Aim: This retrospective study was conducted to define the clinical profile, disease pattern, and treatment outcome of PBL.
Patients and Methods: All patients with PBL diagnosed and treated from 1998 to 2008 at Minia oncology center, Ain Shams oncology department, and Monufia oncology department were retrospectively identified in the Cancer centres Database. Patients were included if they were presented with lymphomatous involvement of the breast as the first manifestation of their disease with no previous diagnosis of any type of Non-Hodgkin's Lymphoma (NHL). All patients underwent a staging workup including computed tomography (CT) scan of the chest, abdomen, and pelvis, as well as bilateral bone marrow biopsies
Results: A total of 20 patients were newly diagnosed with PBL. 1 pt was excluded, because she had primary bony NHL and was treated one

year before developing breast lymphoma. 19 pts were eligible (1 male and 18 female) Median age was 54 (Age>60 was 31.6%), with 68.4% of patients presented with breast mass with Median tumor size 5cm (range 3−6 cm). 31.6% presented with diffuse breast mass with clinical picture of inflammatory disease, they were clinically thought to have a primary breast carcinoma. The diagnosis of lymphoma was made by excisional, inscional, and repeated FNAB biopsy in 52.6%, 10.5%, and 36.8% respectively. Diffuse large B cell lymphoma (DLBCL) was the most common histological subtype seen in 13 pts (68.4%) patients. 5 pts had MALT lymphoma and Mantle cell type was seen in 1 pt (26.3% and 5.3% respectively). Left side involvement was 57.9%. Axillarly LN was detected in 47.4%. LDH was elevated in 68.4%. Stage IE/IIE was 78.9%. 2 pts had BM involvement (10.5%). Mastectomy was done in 26.3% and 60% of them relapsed. 17 pts received chemotherapy (Median 6 cycles (range 0−8) and 15 pts received combined chemo and radiotherapy. Median radiotherapy dose 30 Gy (range 20−40 Gy). Overall response (ORR) was 84.2%, CR/CRU 68.4% with a median follow up 2.4 years (range 0.3−9.7). 9 pts (36.8%) relapsed locally and systemically. 2 pts (10.5%) had a CNS relapse 2 leptomeningeal involvement. The median time to CNS relapse was 13.2mos (8.4−18 mos). In univariate analysis (LDH ($p = 0.02$), IPI ($p = 0.09$), type of relapse ($p = 0.01$) and pathology type ($p = 0.8$). Actuarial 5y Overall Survival (OS) was 56.3% and progression free Survival (PFS) was 51.4%.

Conclusion: PBL can be successfully treated by limited surgery, chemotherapy and radiotherapy. CNS relapse was observed in our series of patients. Rituxmab and CNS prophylaxis should be considered in prospective clinical trial.

Keywords: Primary breast lymphoma, presentation, and treatment outcome.

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Burkitt's lymphoma-derived cells are sensitive targets for the oncolytic activity of rat parvovirus H-1PV

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Background: Oncolytic viruses are recently emerging as promising candidates for the treatment of human cancers. The rat parvovirus H-1PV has been shown to selectively kill human cancer-derived cell lines *in vitro* and to suppress rat and human tumors in animal models. Previous studies have shown that some transformed B cells are H-1PV targets *in vitro*. The present study aims at investigating, both *in vitro* and *in vivo*, H-1PV potential as a therapeutic agent against human lymphoma.

Materials and Methods: A panel of Burkitt's lymphoma (BL)-derived cell lines was used to study H-1PV-induced oncolysis *in vitro*. Infectious parvovirus progeny production was measured by a modified plaque assay. Immunofluorescent staining of lytic Epstein-Barr virus (EBV) proteins was used to detect EBV latency break. Propidium iodide/Annexin-V staining of H-1PV-infected cells was performed to reveal the type of death. For the *in vivo* studies, BL cells were engrafted in SCID mice. Tumor-bearing animals received a single intratumoral H-1PV dose at either early or late time-points after tumor initiation. RT-PCR and Southern blotting were used to detect H-1PV expression and replication in treated tumors.

Results: BL-derived cells were found to be highly sensitive H-1PV targets *in vitro*, irrespective of EBV persistence or rituximab resistance. Parvovirus-infected cells died through necrosis, and death was not due to lytic EBV replication since no signs of EBV latency break could be observed. BL cells supported a productive H-1PV infection. In contrast, normal healthy donor B lymphocytes were not permissive for H-1PV infection. *In vivo*, complete tumor regression accompanied with long-term survival was observed, even when the parvovirus was applied at advanced stages of lymphoma development. H-1PV DNA replication and extensive necrosis, correlating with accumulation of viral cytotoxic NS1 proteins, were observed in regressing tumors.

Conclusions: Altogether, *in vitro* and *in vivo* data suggest that H-1PV deserves to be further considered as a candidate for the treatment of human non-Hodgkin B-cell lymphoma.

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A subset of chemotherapy-refractory diffuse large B-cell lymphomas is characterized by constitutive upstream activation of the intrinsic apoptosis pathway

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Background: Inhibition of the apoptosis cascade is an important cause of therapy resistance in diffuse large B-cell lymphomas (DLBCL). In this study, we investigated the functionality of the intrinsic apoptosis pathway in lymphoma cells of thirty DLBCL biopsies.

Materials and Methods: DLBCL patient samples were investigated for their expression levels of apoptosis-related genes using reverse transcription-multiplex ligation-dependent probe amplification (RT-MLPA) analysis. Functional analysis of the intrinsic, caspase-9-mediated pathway was done using fluorescence-activated cell sorting analysis, Western blot analysis, and immunohistochemistry.

Results: Two DLBCL groups were identified using RT-MLPA analysis; one group with low expression levels of both pro- and anti-apoptotic genes and one group (54% of the DLBCL) with high expression levels of these genes. DLBCL with high expression levels of pro- and anti-apoptotic genes frequently appeared to be refractory to clinical chemotherapy. Functional analysis in these latter DLBCL samples and DLBCL cell lines with comparable expression profiles revealed high levels of spontaneous caspase 9 activity, mitochondrial membrane depolarization and release of cytochrome c in the cytosol, without induction of apoptosis, indicating disruption of the apoptosis pathway downstream of caspase 9 activation. Furthermore, high levels of p53 were found in most of these DLBCL patient samples and DLBCL cell lines. Upstream inhibition of the intrinsic pathway with a p53-inhibitor resulted in a decrease in caspase 9 activity in DLBCL cell lines.

Conclusions: We conclude that the intrinsic caspase 9-mediated apoptosis pathway may be constitutively activated with concomitant downstream inhibition of the convergence apoptosis pathway in chemotherapy-refractory DLBCL. Constitutive caspase 9 activation might be caused by stabilization of p53 expression.

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Association of polymorphisms in cytokine genes with Diffuse Large B Cell Lymphoma and its outcomes in Omani Arabs

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Background: The study was carried out to see whether polymorphisms in cytokine genes were associated with Diffuse Large B cell Lymphoma (DLBCL) in Omani Arab patients. Additionally, we studied whether these polymorphisms correlated with either the prognostic features at presentation, or with overall survival.

Patients and Methods: Over the study period of 5 years, a total of 84 patients with DLBCL were evaluable, the DNA was examined for mutations in IL-10 (T-3575A) and TNF-α (G-308A) genes using PCR, and restriction fragment length polymorphism (RFLP). DNA was also extracted from 115 age and gender matched controls. Clinical data were extracted from the clinical database, and the associations were studied using chi-square test. Correlation with survival was studied using the method of Kaplan and Meier. The study was approved by the Medical research and Ethics Committee.

Results: Median age was 47 years, and there were 46 males and 34 females. Homozygous form of IL-10 (T-3575A) was associated with DLBCL (OR 3.181; 95% CI 1.020−9.926; $p = 0.046$), whereas, no association was found with TNF-α (G-308A) polymorphism. TNF-α (G-308A) was strongly associated with advanced stage of the disease ($p = 0.009$). Also the combination of mutant form of IL-10 and TNF-α was associated with an advanced stage of the disease ($p = 0.026$). Heterozygous form of IL-10 (T-3575A) was associated with a poor overall survival (median survival 22 months versus 60 months), whereas, polymorphic TNF-α (G-308A) gene did not affect the overall survival.

Conclusion: Homozygous form of mutant IL-10 gene may be implicated in the genesis of DLBCL in Omani patients. Mutation in TNF-α gene was associated with advanced stage, whereas, mutation in IL-10 gene was associated with inferior survival.