Sultan Qaboos University Med J, February 2014, Vol. 14, Iss. 1, pp. e50-58, Epub. 27TH Jan 14
Submitted 13TH May 13
Revision Req. 2ND Jul 13; Revisions Recd. 1ST Aug 13
Accepted 25TH Aug 13

CLINICAL & BASIC RESEARCH

The *p53* Mutation/Deletion Profile in a Small Cohort of the Omani Population with Diffuse Large B-Cell Lymphoma

*Yahya Tamimi,[1] Sheikha Al-Harthy,[1] Ibrahim Al-Haddabi,[2] Mohammed Al-Kindi,[1] Hamza Babiker,[1] Mansour Al-Moundhri,[3] Ikram Burney[4]

تشكيل طفرات/عمليات الحذف ل *p53* في مجموعة صغيرة من العمانيين العمانيين المصابين بالأورام الليمفاوية كبيرة الحجم للخلايا البائية

يحيى التاميمي، شيخة الحارثي، إبراهيم الهدابي، محمد الكندي، حمزة بابكر، منصور المنذري، إكرام بيرني

ABSTRACT: ***Objectives:*** Mutations/deletions affecting the *TP53* gene are considered an independent marker predicting a poor prognosis for patients with diffuse large B-cell lymphoma (DLBCL). A cohort within a genetically isolated population was investigated for *p53* mutation/deletion status. ***Methods:*** Deoxyribonucleic acid (DNA) samples were extracted from 23 paraffin-embedded blocks obtained from DLBCL patients, and subjected to polymerase chain reaction (PCR) amplification and sequencing of exons 4–9 of the *p53* gene. ***Results:*** While 35% of patients analysed displayed allelic deletions (P <0.01), immunohistochemical analysis revealed a mutation rate of 69.5%. It is noteworthy that the rate of *p53* mutations/deletions in this small cohort was found to be higher than that previously reported in the literature. Interestingly, patients with *p53* mutations displayed a better overall survival when compared to those without. The survival of patients treated with rituximab-containing combination chemotherapy was significantly better than those who did not receive rituximab (P <0.05). Furthermore, a modelling analysis of the deleted form of *p53* revealed a huge structural change affecting the DNA-binding domain. ***Conclusion:*** The *TP53* mutation/deletion status plays a role in mechanism(s) ruling the pathogenesis of DLBCL and may be useful for stratifying patients into distinct prognostic subsets.

Keywords: Mutations; Gene Deletion; Lymphoma, B-Cell; Paraffin Embedding; Immunohistochemistry; Oman.

الملخص: **الهدف**: تعتبر الطفرات/عمليات الحذف التي تمس *TP53* علامة مستقلة للتنبؤ السيء للمرضى المصابين بالأورام اللمفاوية كبيرة الحجم للخلايا البائية المنتشرة (DLBCL). تم اختبارالطفرات/عمليات الحذف للجين *p53* لعينة من المرضي المعزولين وراثيا والذين تم اختيارهم لهذه الدراسة. **الطرق**: تم استخراج مادة الحمض النووي (DNA) من 23مريض والتي عرضت للمضاعفة عن طريق تفاعل البلمرة المتسلسل ومن ثم تم ايجاد التسلسل النيوكليوتيدي للأكسونات 9–4 من الجين *p53*. **النتائج**: في حين أن 35% من المرضى الذين تم تحليلهم أظهروا حذفا أليليا (P <0.01)، كشف التحليل المناعي – النسيجي معدل تحور بنسبة 69.5%. ومن الجدير بالذكر أن معدل الطفرات/عمليات الحذف للجين *p53* في العينة التي تم دراستها وجدت أعلى مما هو موجود في الدراسات السابقة. كما أنه من المثير للاهتمام أن المرضى الذين يحملون طفرات ب–*p53* أظهروا فرص في البقاء علي قيد الحياة بنسب أفضل عند مقارنتهم بغيرهم من المرضى الذين لا يحملون هذه الطفرات. أظهرت الدراسة أيضاً أن البقاء على قيد الحياة للمرضى الذين يعالجون بعقار الريتوكسوماب العلاج الكيميائي المر كب المحتو ي أساسا علي الر يتو كسو ماب كان أفضل عند مقارنتهم بأولئك الذين لم يتلقوا الريتوكسيماب.(P <0.05) علاوة على ذلك كشف تحليل النمذجة للحذف في *p53* تغييرا هيكليا كبيرا يؤثر في مجال ربط الحمض النووي DNA. **الإستنتاج**: نستنتج أن الطفرات/عمليات الحذف ل *p53* تلعب دورا في الآليات التي تحكم التسبب ب DLBCL كما أنها يمكن أن تكون مفيدة لتقسيم المرضى إلى مجموعات فرعية للتنبؤ بحالة المرض.

مفتاح الكلمات: طفرات وراثية؛ عمليات الحذف للجين؛ الأورام الليمفاوية؛ الخلايا الليمفاوية البائية؛ التكتل بالبارافين؛ التحليل المناعي النسيجي؛ عمان.

Advances in Knowledge

- *The TP53 mutation/deletion profile in the present study revealed a role in the mechanism(s) ruling the pathogenesis of diffuse large B-cell lymphoma (DLBCL) that might be useful for stratifying subpopulations based on their different genetic backgrounds.*

Application to Patient Care

- *The findings shed some light on the role of p53 mutations/deletions in mechanisms involved in the pathogenesis of DLBCL, which may help in stratifying affected patients into distinct prognostic subsets and in providing tailored therapies.*

Departments of [1]Biochemistry and [3]Medicine, College of Medicine & Health Sciences, Sultan Qaboos University; Departments of [2]Pathology and [4]Medicine, Sultan Qaboos University Hospital, Muscat, Oman

**Corresponding Author e-mail: yahyatam@squ.edu.om*

Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin's lymphoma (NHL) in developing countries, including those in the Middle East, where it may constitute up to 60% of cases.[1] It has been shown that DLBCL is morphologically a very heterogeneous disease and the affected patients usually present with an advanced stage of the disease.[1–3] Because of this complex heterogeneity, there is an unmet need to identify prognostic markers to help discriminate between DLBCL subgroups.

The p53 protein encoded by the *p53* gene is a tumour suppressor gene playing a crucial role in cell cycle control, cell growth, apoptosis and senescence, and in the response to stress signals such as deoxyribonucleic acid (DNA) damage and hypoxia.[4,5] Mutations of the *p53* gene are common and have been implicated in the disease progression of more than 50% of epithelial cancers.[6]

The normal functioning of the *p53* gene is important for the eradication of tumours.[7] *TP53* mutations are responsible for an increased resistance to chemotherapy, a decrease in apoptosis, neoangiogenesis and the early progression of the disease leading to a shortened overall survival rate.[8] In lymphoid malignancies, the incidence of *p53* mutations is reported to have a range of 5–25% of cases, the majority of which (~90%) are clustered in the DNA-binding domain of the protein.[6–8] Other changes, such as single-nucleotide polymorphisms (SNPs), allelic loss and complete deletions between exons 4–9, have been also described.[9]

The prognostic value of *TP53* mutations has not been consistent in DLBCL, where *TP53* mutations are considered a poor prognostic factor.[8,10–12] This inconsistency is likely due to the *TP53* mutation's heterogeneity, the limit of mutation detection methods or the diversity in the *TP53* mutation's functions.[13,14]

Rituximab, an anti-CD20 antibody, has improved the survival of patients with DLBCL significantly when combined with cyclophosphamide, hydroxydaunorubicin, oncovin and prednisolone (CHOP) chemotherapy, and acts through complement-mediated cytotoxicity and antibody-dependent cellular cytotoxicity.[15] High doses of rituximab inhibit cell growth through intracellular calcium (Ca2+) mobilisation. This is independent of the CD20 antigen and the inhibition of downstream key effectors such as protein 38, nuclear factor kappa-light-chain-enhancer of activated B cells, extracellular signal-regulated kinase, protein kinase B, and the downregulation of cytokine interleukin-10 and B-cell lymphoma-2 (BCL-2).[15–17] Rituximab and other drugs can generate different stresses, affecting *TP53* functioning. Similarly, the *p53*-independent signalling pathways induced by rituximab can affect the transcription activity of the *p53* gene. Thus, this lends validity to the importance of exploring the prognostic value of *PT53* mutation/deletion in patients with DLBCL treated with different drugs.

Studies on patients of Arab ethnicity in the Middle East describing *p53* aberrations in DLBCL diseases are scarce; thus, this study aims to address the prognostic significance of *p53* mutation/deletions in a small cohort with DLBCL. Exons 4–9 of the *p53* gene were analyzed using polymerase chain reaction (PCR), sequencing and immunohistochemical analysis on archival paraffin-embedded material, and the mutation/deletion status was correlated with the clinical outcome. Additionally, molecular modelling was used to estimate the structural changes in the affected *TP53* protein.

Methods

This study was approved by the Medical Ethics Research Committee of the College of Medicine & Health Sciences at Sultan Qaboos University, Muscat, Oman. Paraffin-embedded formalin-fixed tissue samples were obtained from Sultan Qaboos University Hospital. All of the samples were from Omanis diagnosed with stage IIB DLBCL or higher, according to the Ann Arbor system. The II stage indicates that either two or more lymph node regions on the same side of the diaphragm or one lymph node region and a contiguous extralymphatic site were involved, and the classification B indicates the presence of systemic symptoms. All the patients were treated between January 2001 and September 2008. Table 1 outlines the histopathology and clinical data.

Paraffin blocks from 23 patients were examined by a pathologist and areas containing at least 95% cancer cells were marked and cut for DNA extraction. Serial sections of 8 µm were cut, deparaffinised in xylene and rehydrated in

Table 1: Clinical/pathological features of the cohort and aberrations in the *p53* gene

Age in years and gender	IPI category	Stage	B symptoms	Site of the tumour	IHC staining score	Sequencing and LOM	Status
57 F	4	4B	Yes	Extra-nodal	3	No mutation	Dead
50 M	1	3B	No	Extra-nodal	2	No mutation	Alive
20 M	3	4B	Yes	Extra-nodal	2	LOM in exons 4, 5, 7 and 8	Alive
47 F	2	3A	No	Nodal	2	LOM in exons 4 and 7–9	Alive
60 F	-	4B	Yes	Extra-nodal	3	No mutation	Dead
25 F	3	4B	Yes	Extra-nodal	3	LOM in exons 4, 5 and 7–9	Alive
46 F	1	2B	Yes	Nodal	-	LOM in exons 4, 5 and 7–9	Alive
70 M	3	3B	Yes	Nodal	1	No mutation	Alive
66 M	4	3B	Yes	Nodal	3	No mutation	Dead
50 F	3	3A	No	Extra-nodal	3	Mutation in exon 5	Dead
70 M	4	4B	Yes	Nodal	2	No mutation	Dead
65 M	-	4B	Yes	-	1	No mutation	Dead
53 M	1	2B	-	-	1	LOM in exons 4, 5 and 7–9	Alive
62 M	4	3B	Yes	Nodal	0	No mutation	Dead
65 M	5	4A	Yes	Extra-nodal	2	No mutation	Dead
48 M	4	4B	-	Extra-nodal	0	LOM in exons 4 and 7–9	Alive
21 F	-	-	-	Nodal	2	LOM in exons 4–9	Alive
21 F	3	2B	Yes	Extra-nodal	2	No mutation	Alive
59 M	-	4B	Yes	Nodal	2	No mutation	Dead
29 M	-	-	-	-	-	-	-
29 M	-	-	-	-	0	No mutation	Alive
30 M	-	-	-	Nodal	0	Mutation and deletion in exon 5	Alive
70 M	5/5	4B	Yes	Extra-nodal	0	No mutation	Alive

IPI = International Prognostic Index; B = presence of systemic symptoms; IHC = immunohistochemistry; LOM = loss of deoxyribonucleic acid material; F = female; M = male; IPI categories: Low risk (0–1 points) - 5-year survival 73%; Low-intermediate risk (2 points) - 5-year survival 51%; High-intermediate risk (3 points) - 5-year survival 43%; High risk (4–5 points) - 5-year survival 26%.

Stage II = cancer located in two separate regions, an affected lymph node or organ and a second affected area, and that both affected areas are confined to one side of the diaphragm.

Stage III = cancer has spread to both sides of the diaphragm, including one organ or area near the lymph nodes or the spleen.

Stage IV = diffuse or disseminated involvement of one or more extralymphatic organs, including any involvement of the liver, bone marrow, or nodular involvement of the lungs.

A = absence of constitutional (B-type) symptoms; B = presence of constitutional (B-type) symptoms.

decreasing ethanol concentrations of 100%, 70% and 50%. A digestion step in 20 mg/ml of proteinase K (Roche Diagnostic GmbH, Mannheim, Germany) was subsequently performed at 55 °C for at least 10 hours. The tissues were then incubated overnight at 55 °C in a lysis buffer solution (Tris(hydroxymethyl) aminomethane pH 6.8, 20% sodium dodecyl sulfate (SDS) and 2% glycerol) (Fermentas, Thermo Fisher Scientific, Vilnius, Lithuania) and subjected to phenol chloroform extraction and ethanol-

DNA precipitation. The DNA concentration was estimated with the NanoDrop method using a ND-1000 spectrophotometer (NanoDrop Products, Thermo Scientific, Wilmington, Delaware, USA). Due to the extremely low concentration of normal cells present in each section composed of more than 90% of cancer cells, normal tissue could not be included for the control of sequencing reactions. This was corrected by taking sequences from normal blood as a reference for the putative polymorphisms.

The extracted DNA was amplified by PCR using a set of designed primers covering the relevant areas of the *p53* gene, including exons 4–9. An additional pair of primers was used to amplify the housekeeping gene *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) and to check for DNA integrity [Table 2]. In order to avoid primer mismatches that might result in negative PCR amplifications, all primers were checked for eventual mutations on their corresponding site. PCR reactions were performed in a total volume of 50 µl using optimised PCR conditions. Each cycle was run through a denaturing step at 94 °C for 55 secs, an annealing step ranging between 55–60 °C for 55 secs (depending on the primers' melting temperatures) and an elongation step of 55 secs at 72 °C. The PCR reactions were run in a thermocycler for 25 cycles, preceded by a denaturing step of 4 mins and followed by an extended elongation time of 7 mins at 72 °C. All amplification reactions were repeated at least twice to confirm the consistency of the PCR reactions.

Specific bands corresponding to the amplified PCR products were purified using the ExoSAP-IT® enzyme clean-up method (Affymetrix, Inc., Santa Clara, California, USA), and the sequencing reactions were initiated using a BigDye Terminator (Applied Biosystems, Inc., Foster City, California, USA). After the ethanol precipitation, the DNA was resuspended in 10 µL of deionised formamide, covered and loaded into the sequencer ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems). Sequences were analysed using the SequencherTM 4.7 software (Gene Codes Corporation, Ann Arbor, Michigan, USA).

For immunohistochemical staining, sections of 4 µm were cut and mounted on polylysine-coated slides, dewaxed and placed in sodium perborate (0.2 M of disodium hydrogen phosphate, 0.3 M of monopotassium phosphate and 0.01 M of sodium chloride, at pH 9), then heated for 2–3 mins at 100 °C in a microwave oven to mediate antigen retrieval. The machine was set up so that heating was controlled in order to ensure only the antigen retrieval, without causing tissue damage. A mouse monoclonal anti-human p53 antibody, recognising both the wild-type and the mutant p53 protein, was applied to the sections. The secondary antibody was an anti-mouse polyclonal conjugated to horseradish peroxidase (Dako A/S, Glostrup, Denmark) and used at 1/5000 dilution. The slides were visualised using the Universal Dako LSAB®+ Kit (Code K 0679, Dako A/S) and scored by a pathologist. The positive cells were characterised by a brown precipitate at the cytoplasmic level as indicated by arrows in Figure 1B, whereas the negative cells were distinguished by their nuclear blue haematoxylin staining and an uncoloured cytoplasm. A cut-off value of p53 staining was set at 20% positive cells. Cases with no staining or less than 20% positive cells at x 400 magnification were scored zero. Cases of weak staining at x 400 magnification (more than 20% positive cells) were scored one, cases of moderate staining at x 100 magnification were scored two, and finally a score of three was given to cases with any percentage of staining recognisable at x 40 magnification. Sections displaying positive immunohistochemistry (IHC) staining were considered equivalent to *p53* mutations.

Clinicopathological variables such as age, gender, clinical stage, performance status, serum lactate dehydrogenase (LDH) levels and the site of disease were obtained from clinical reports. The number of extranodal sites was recorded and the patients were categorised using the International Prognostic Index (IPI). Overall survival was calculated from the time of diagnosis to death or until February 2011 using the Statistical Package for the Social Sciences (SPSS), Version 19 (IBM, Corp., Chicago, Illinois, USA) software. The minimum follow-up period was 30 months and the maximum was nine years. Median survival was estimated using the Kaplan-Meier method, and the log-rank test (Mantel-Cox test) was used for comparison among the different groups. The Fisher's exact test was applied to estimate the putative association of mutations to the disease. One of the major limitations of this study was the small size of the cohort; the statistical methods used were therefore

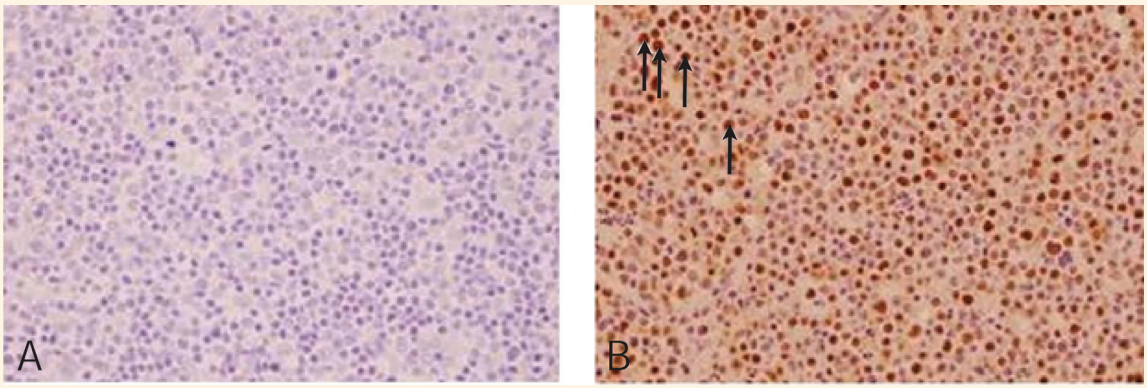

Figure 1 A & B: A representative example of the immunohistochemistry showing the positive and the negative staining obtained for (**A**) samples and (**B**) controls, respectively. The arrows indicate the stained cells at a magnification of x 40.

carefully selected for small population sizes to avoid incorrect conclusions.

Results

Paraffin blocks were available from 23 patients (15 males and 8 females) with a median age at diagnosis of 49 years (range: 20–70 years). A complete clinical profile was available for 19 of the 23 patients only. Out of these 19 patients, two had stage IIB (as described previously) DLBCL, eight had stage III (indicating the involvement of lymph node regions on both side of the diaphragm), and nine had stage IV of the disease (indicating disseminated involvement of one or more extralymphatic organs). Using the IPI, two patients had a low to intermediate risk, whereas eight patients had a high to intermediate risk and nine patients a high risk [Table 1].

Out of the 21 patients with IHC staining scores, five displayed negative, three displayed weakly positive (score = 1), eight displayed moderately positive (score = 2) and five displayed strongly positive staining (score = 3). Figure 1 shows the p53 staining at different magnifications. Table 1 summarises the IHC and sequencing data with the location of the aberration (mutation/deletion) and the DLBCL origin. PCR conditions were optimised using DNA extracted from healthy donor blood samples. All of the tested exons displayed single bands of an expected size, implying the specificity of the PCR reactions. The housekeeping gene *GAPDH* was run in parallel to verify the integrity of the DNA. The optimised conditions were applied to amplify the DNA samples [Figure 2]. A gel examination revealed extracted DNA of lower molecular weights and, therefore, it was difficult to amplify the large fragments (≥400 base pairs [bp]). Thus, primers generating lower size products were designed for both *p53* and *GAPDH*. The PCR products were resolved using 1% agarose gel electrophoresis and all of the samples displayed specific bands of the expected sizes in exons 4, 5, 6 and 9. However, exon

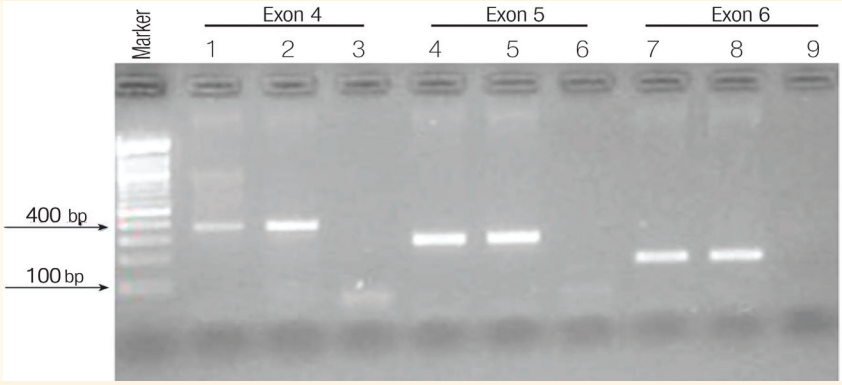

Figure 2: The optimisation of the polymerase chain reaction (PCR) conditions for exons 4–6 of the *p53* gene. The polymerase chain reaction amplification products of exon 4 (lanes 1 and 2), exon 5 (lanes 4 and 5) and exon 6 (lanes 7 and 8) of the *p53* gene resolved on 1% agarose gel electrophoresis and compared to the 100 base pairs (bp) deoxyribonucleic acid marker (lane 1). Samples were run in duplicate, including the negative controls (lanes 3, 6 and 9).

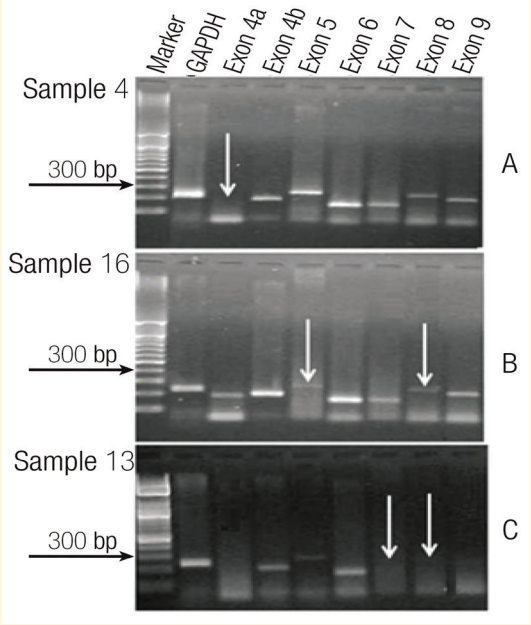

Figure 3 Panels A, B & C: The representative gel displaying the expression of the *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) gene and exons 4–9 of the *p53* gene in samples 4, 13 and 16, respectively. The arrows indicate the obtained deletions (**A & C**) and partial loss (**B**). The partial loss is similar to gene dosage, where substantial deoxyribonucleic acid damage is obvious but not completely lost, likely resulting in far-reaching repercussions on gene function.

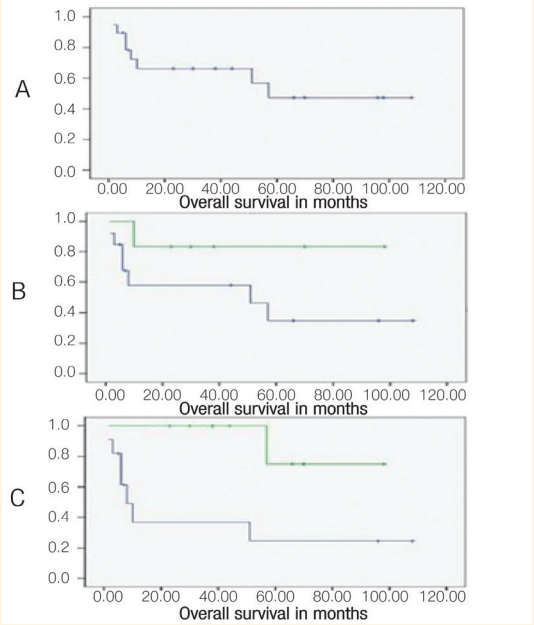

Figure 4 A, B & C: The median overall survival curve. **A:** The overall survival plot displays the 57 months recorded for the studied cohort, while the five-year survival rate observed was 48%. **B:** However, the overall survival curve shows a trend towards better overall survival in patients who harboured mutations in the *p53* gene, as analysed by sequence analysis. The immunohistochemistry analysis revealed no statistically significant difference between the survival of those with or without *p53* mutations. **C:** The overall survival curve shows that patients receiving rituximab in addition to cyclophosphamide, hydroxydaunorubicin, oncovin and prednisolone (R-CHOP) did significantly better than those who only received CHOP (P <0.05).

8 revealed either a complete absence of signals or faint bands (partial loss).

In the current study, eight samples highly amplified the *GAPDH* gene, but not the *p53* gene, suggesting the presence of putative deletions [Figure 3A–C]. To confirm this observation, samples were amplified from the *OVCA2* gene located adjacent to the *p53* gene on chromosome 17 and specific bands were obtained suggesting that the deletion is specific for the *p53* gene. Moreover, in addition to the putative deletions observed, samples showing a high level of *GAPDH* but weak bands for the amplified exons 4–9 were reported, representing typical examples of the partial loss of genetic material as shown in Figure 3B. The electropherogram revealed missense mutations in codon 143, changing the amino acid valine (non-polar) to leucine (non-polar) (GTG to TTA). In codon 145, missense mutations caused a change from the leucine (non-polar) to threonine (polar) amino acid (CTG to ACG). In codons 146 and 150, there was a change from the tryptophan (non-polar) to the basic arginine (TGG to AGG) and from the threonine (polar) to arginine (ACA to AGA), respectively. Changing amino acids with different polarities may affect the functional profile of the mutated p53 protein.

The amino acid sequences of the p53 protein carrying the *p53* mutation *W150R* and the sequence harbouring the deletion in exon 5 were subjected to a modelling analysis to predict the changes that occur in the p53 structure. Both aberrations (mutation and deletion) displayed an obvious change within the p53 three-dimensional structure (data not shown).

While the five-year survival rate was 48%, the median overall survival time was 57 months [Figure 4A]. The correlation of survival with the data obtained using IHC did not demonstrate any statistically significant difference between those with or without *p53* mutations. However, patients with *p53* mutations detected by sequencing demonstrated a trend of better overall survival compared to those who did not harbour mutations [Figure 4B]. Of the patients, 11 out of 19 received

a combined therapy consisting of CHOP, whereas eight patients received the anti-CD20 antibody, rituximab, in addition to CHOP (R-CHOP). The survival of patients receiving R-CHOP was significantly better than those who received CHOP (P <0.05), as shown in Figure 4C.

Discussion

In this study, the entire region of the *p53* gene, including exons 4–9, was screened for the presence of mutations/deletions; partial losses as well as deletions in eight of 19 patients were identified. To validate this observation, the *OVCA2* gene localised in the neighbouring area of the *p53* gene on chromosome 17 was amplified. While no amplification of the *p53* gene could be obtained using the p53 primers, specific bands were obtained systematically when the OVCA2 primers were used. Moreover, the p53 primers' targeted area was meticulously scanned for the presence of any polymorphisms that might hamper the appropriate binding of the primers, resulting in non-amplified amplicons; however, no aberration was found. Southern blotting would have been more appropriate to confirm the deletions and loss of genetic material. However, the amount of DNA required for a Southern blot is substantial (more than 5 mg), and therefore difficult to obtain from archival material. Alternatively, confirming deletions by comparison of the housekeeping gene amplification of *GAPDH* with PCR products from the exons of interest is widely used.[18]

Faint bands were obtained by amplifying exons 4, 5, 7 and 8 using the DNA from a patient with stage IV t-cell-rich DLBCL (t-DLBCL), suggesting that the loss of p53 material may be associated with the transformation of the disease. Another patient with stage IIB DLBCL displayed two faint bands for exons 5 and 6, while complete deletions were observed in exons 4, 7, 8 and 9 [Figure 3A–C]. Both patients displayed a positive staining by IHC. These data are in agreement with previous reports indicating that up to 20% of DLBCL patients harbour deletions on the short arm of chromosome 17 (chromosome 17p13) corresponding to the location of the *p53* gene.[19] At the structural level, both the *W150R* mutation and the deletion in exon 4 displayed substantial changes in the *p53* gene when compared to the wild-type molecule. Changing the non-polar amino acid tryptophan (W) at position 150 by the larger polar arginine amino acid (R), within a well-conserved region (from amino acid 101 to 306) containing the DNA-binding domain, would likely alter the tumour-suppressing function of the p53 protein. The predicted model of the p53-exon-4-deleted form revealed an obviously disorganised structure of the p53 protein affecting several domains, including the transactivation and the binding domains.

Both the quantitative PCR and Southern blotting methods have revealed deletions in relevant genes in DLBCL such as *CDKN2* and *CDKN2B*.[20] Moreover, a substantial decrease in messenger ribonucleic acid (mRNA) expression levels associated with specific gene signatures has been confirmed with gene deletion.[21] Interestingly, these deletions were directly associated with a poor prognosis in DLBCL.[20–23] A deletion on the short arm of chromosome 17 may be a sign of transformation from other types of lymphoma to DLBCL.[19] Moreover, it was reported that *p53* mutations and deletions are detected in t-DLBCL and predict resistance to treatment and short survival in variants of DLBCL.[24,25] The transformation of follicular lymphoma can evolve through a variety of mechanisms characterised by a high proliferation rate and the mutation of the *p53* gene, amplification of the *REL* gene, loss of *CDNK2A* and changes in the *c-myc* gene expression.[26]

Missense mutations in exon 5 were found in two patients, one of whom presented with extranodal t-DLBCL at stage III of the disease. The IHC analysis showed a positive staining, which is consistent with sequence analysis data. The second patient had a similar pattern with deletions in exons 4, loss of material in exon 7 and a C to T point mutation in exon 5, changing the proline amino acid to serine at position 177 (P 177 S). No positive staining, however, was revealed by IHC. This could be explained by a large deletion in the *p53* gene that may hamper transcription. The missense mutation found in exon 5 was located on the DNA-binding domain of the p53 protein, which is divided into three loops (L1, L2 and L3) and two loop sheet helix (LSH) regions. L1, L3 and LSH make direct contact with the DNA while L2 is required for the folding and stabilisation of the DNA-binding domain, with no direct contact with the DNA.[8] The missense mutation on exon 5 is located in the core domain within L2 and L3. Therefore, this could affect both

the DNA binding and the folding process of the related domain. It was found that mutations within areas of direct contact with the DNA resulted in a poor survival rate, while mutations that affected the folding of the protein had no significant impact on the patient's overall survival.[8]

Positive staining was shown in ten patients by IHC; however, no aberrations were detected by sequencing. This contradiction is likely due to the limited area covered by the sequencing, omitting some exons (for instance exons 1–3) as well as the promoter area, also frequently targeted by mutations. Moreover, the IHC technique cannot be used alone to predict mutations since the mechanisms responsible for gene overexpression may generate false-positive results.[27] On the other hand, protein-stabilising factors such as the MDM2 protein could have an effect by promoting the rapid degradation of the phosphorylated form of *p53*.[28] The *p53* gene activates the expression of MDM2 in an auto-regulatory feedback loop. Therefore, mutations affecting MDM2 cause a half-life increase of the p53 protein and its accumulation, allowing its detection by IHC.[29]

Unexpectedly, a better overall survival rate for patients harbouring *p53* mutations was observed when compared with patients with no mutations in the *p53* gene. Data concerning this matter are conflicting.[9,11,30,31] For instance, Ichikawa *et al.* demonstrated that the overall survival was inferior for an entire cohort with *p53* mutations. However, this effect was not seen in patients with a high-risk IPI category.[11] In the current study, at presentation all but two patients had a high to intermediate and high risk IPI category. An alternative explanation could be related to the effect of treatment. As shown in Figure 4C, patients treated with R-CHOP had a higher overall survival compared to those who were treated with CHOP alone. The *p53*-induced apoptotic pathway could be overhauled as a result of mutations in the *p53* gene. Conversely, rituximab inhibits the anti-apoptotic protein BCL-2, thus causing apoptosis induction. It is plausible that rituximab-induced apoptosis may override the mutant *p53*-mediated anti-apoptotic pathway. Similarly, Hussein *et al.* showed a negative correlation between BCL-2 and p53 protein expression in lympho-proliferative disorders.[32] In a recent report, Xu-Monette *et al.* studied the prognostic significance of *p53* mutations in DLBCL using a large cohort of *de novo* DLBCL patients treated with R-CHOP, and showed that those with *TP53* mutations had worse overall and progression-free survival compared to those without.[33] It is worth noting that the majority of studies on the association of *p53* with prognosis in DLBCL are from the pre-rituximab era.

Data reporting on the DLBCL incidence in the Middle East and comparisons to those in Western countries, including North America, are scarce. However, Ameen *et al.* studied NHL frequency among different ethnic groups in Kuwait and compared those results with studies from the Western world. They found that the Kuwaiti population had a higher prevalence of DLBCL and extranodal presentation.[34] Despite the small number of patients recruited, the data on Omani patients in this study are in agreement with the increase of DLBCL frequency in the Middle East region; the aberrations (mutation/deletion) of the *p53* gene might be directly linked to this higher frequency.

Despite several tentative attempts, we were unable to increase the number of samples used for this study which was a major limitation. Precautions were taken throughout and the small size of the sample was taken into account by using the appropriate statistical methods.

Conclusion

Mutations/deletions were frequently detected within the relevant region of the *p53* gene. The incidence of mutations was higher than those previously reported in the literature and may suggest that the biology of the disease changes depending on ethnicity. The survival data, especially with regards to potential treatment, are intriguing and require verification in a larger cohort. The number of patients recruited for this study was very modest and analysis should be extended to a large cohort in order to draw appropriate conclusions.

ACKNOWLEDGEMENT

This work was supported by the Postgraduate Studies & Research Programme at Sultan Qaboos University, Muscat, Oman.

References

1. Müller AM, Ihorst G, Mertelsmann R, Engelhardt M.

Epidemiology of non-Hodgkin's lymphoma (NHL): Trends, geographic distribution, and etiology. Ann Hematol 2005; 84:1–12.

2. Naresh KN, Advani S, Adde M, Aziz Z, Banavali S, Bhatia K, et al. Report of an International Network of Cancer Treatment and Research workshop on non-Hodgkin's lymphoma in developing countries. Blood Cells Mol Dis 2004; 33:330–7.

3. Montoto S, Fitzgibbon J. Transformation of indolent B-cell lymphomas. J Clin Oncol 2011; 29:1827–34.

4. Borrás C, Gómez-Cabrera MC, Viña J. The dual role of p53: DNA protection and antioxidant. Free Radic Res 2011; 45:643–52.

5. Hede SM, Nazarenko I, Nistér M, Lindström MS. Novel perspectives on p53 function in neural stem cells and brain tumors. J Oncol 2011; 2011:1–11.

6 Gudkov AV, Komarova EA. The role of p53 in determining sensitivity to radiotherapy. Nat Rev Cancer 2003; 3:117–29.

7. Gaidarenko O, Xu Y. Transcription activity is required for p53-dependent tumor suppression. Oncogene 2009; 28:4397–401.

8. Levine AJ, Vosburgh E. P53 mutations in lymphomas: Position matters. Blood 2008; 112:2997–8.

9. Bittenbring J, Parisot F, Wabo A, Mueller M, Kerschenmeyer L, Kreuz M, et al. MDM2 gene SNP309 T/G and p53 gene SNP72 G/C do not influence diffuse large B-cell non-Hodgkin lymphoma onset or survival in central European Caucasians. BMC Cancer 2008; 8:116.

10. Zenz T, Eichhorst B, Busch R, Denzel T, Häbe S, Winkler D, et al. *TP53* mutation and survival in chronic lymphocytic leukemia. J Clin Oncol 2010; 28:4473–9.

11. Ichikawa A, Kinoshita T, Watanabe T, Kato H, Nagai H, Tsushita K, et al. Mutations of the p53 gene as a prognostic factor in aggressive B-cell lymphoma. N Engl J Med 1997; 337:529–34.

12. Kerbauy FR, Colleoni GW, Saad ST, Regis Silva MR, Correa Alves A, Aguiar KC, et al. Detection and possible prognostic relevance of p53 gene mutations in diffuse large B-cell lymphoma. An analysis of 51 cases and review of the literature. Leuk Lymphoma 2004; 45:2071–8.

13. Xu-Monette ZY, Medeiros LJ, Li Y, Orlowski RZ, Andreeff M, Bueso-Ramos CE, et al. Dysfunction of the TP53 tumor suppressor gene in lymphoid malignancies. Blood 2012; 119:3668–83.

14. Xu-Monette ZY, Young KH. The TP53 tumor suppressor and autophagy in malignant lymphoma. Autophagy 2012; 8:842–5.

15. Weiner GJ. Rituximab: Mechanism of action. Semin Hematol 2010; 47:115–23.

16. Unruh TL, Zuccolo J, Beers SA, Kanevets U, Shi Y, Deans JP. Therapeutic (high) doses of rituximab activate calcium mobilization and inhibit B-cell growth via an unusual mechanism triggered independently of both CD20 and Fcgamma receptors. J Immunother 2010; 33:30–9.

17. Kheirallah S, Caron P, Gross E, Quillet-Mary A, Bertrand-Michel J, Fournié JJ, et al. Rituximab inhibits B-cell receptor signaling. Blood 2010; 115:985–94.

18. Tamimi Y, Bringuier PP, Smit F, van Bokhoven A, Debruyne FM, Schalken JA. p16 mutations/deletions are not frequent events in prostate cancer. Br J Cancer 1996; 74:120–2.

19. Stöcklein H, Smardova J, Macak J, Katzenberger T, Höller S, Wessendorf S, et al. Detailed mapping of chromosome 17p deletions reveals HIC1 as a novel tumor suppressor gene candidate telomeric to TP53 in diffuse large B-cell lymphoma. Oncogene 2008; 27:2613–25.

20. Jardin F, Ruminy P, Kerckaert JP, Parmentier F, Picquenot JM, Quief S, et al. Detection of somatic quantitative genetic alterations by multiplex polymerase chain reaction for the prediction of outcome in diffuse large B-cell lymphomas. Haematologica 2008; 93:543–50.

21. Jardin F, Jais JP, Molina TJ, Parmentier F, Picquenot JM, Ruminy P, et al. Diffuse large B-cell lymphomas with CDKN2A deletion have a distinct gene expression signature and a poor prognosis under R-CHOP treatment: A GELA study. Blood 2010; 116:1092–104.

22. Grønbaek K, de Nully Brown P, Møller MB, Nedergaard T, Ralfkiaer E, Møller P, et al. Concurrent disruption of p16INK4a and the ARF-p53 pathway predicts poor prognosis in aggressive non-Hodgkin's lymphoma. Leukemia 2000; 14:1727–35.

23. Villuendas R, Sánchez-Beato M, Martínez JC, Saez AI, Martinez-Delgado B, García JF, et al. Loss of p16/INK4A protein expression in non-Hodgkin's lymphomas is a frequent finding associated with tumor progression. Am J Pathol 1998; 153:887–97.

24. Simonitsch-Klupp I, Hauser I, Ott G, Drach J, Ackermann J, Kaufmann J, et al. Diffuse large B-cell lymphomas with plasmablastic/plasmacytoid features are associated with TP53 deletions and poor clinical outcome. Leukemia 2004; 18:146–55.

25. Bosga-Bouwer AG, van den Berg A, Haralambieva E, de Jong D, Boonstra R, Kluin P, et al. Molecular, cytogenetic, and immunophenotypic characterization of follicular lymphoma grade 3B; A separate entity or part of the spectrum of diffuse large B-cell lymphoma or follicular lymphoma? Hum Pathol 2006; 37:528–33.

26. Davies AJ, Rosenwald A, Wright G, Lee A, Last KW, Weisenburger DD, et al. Transformation of follicular lymphoma to diffuse large B-cell lymphoma proceeds by distinct oncogenic mechanisms. Br J Haematol 2007; 136:286–93.

27. Kamata H, Mitani S, Fujiwara M, Aoki N, Okada S, Mori S. Mutation of the p53 tumour suppressor gene and overexpression of its protein in 62 Japanese non-Hodgkin's lymphomas. Clin Exp Med 2007; 7:39–46.

28. Koduru PR, Raju K, Vadmal V, Menezes G, Shah S, Susin M, et al. Correlation between mutation in P53, p53 expression, cytogenetics, histologic type, and survival in patients with B-cell non-Hodgkin's lymphoma. Blood 1997; 90:4078–91.

29. Tokino T, Nakamura Y. The role of p53-target genes in human cancer. Crit Rev Oncol Hematol 2000; 33:1–6.

30. El-Bolkainy TN, El-Bolkainy MN, Khaled HM, Mokhtar NM, Eissa SS, Gouda HM, et al. Evaluation of MIB-1 and p53 overexpression as risk factors in large cell non-Hodgkin lymphoma in adults. J Egypt Natl Canc Inst 2007; 19:231–8.

31. Naresh KN, Banavali SD, Bhatia KG, Magrath I, Soman CS, Advani SH. Expression of P53 and bcl-2 proteins in T-cell lymphoblastic lymphoma: Prognostic implications. Leuk Lymphoma 2002; 43:333–7.

32. Hussein MR, Al-Sabae TM, Georgis MN. Analysis of the Bcl-2 and p53 protein expression in the lymphoproliferative lesions in the Upper Egypt. Cancer Biol Ther 2005; 4:324–8.

33. Xu-Monette ZY, Wu L, Visco C, Tai YC, Tzankov A, Liu WM, et al. Mutational profile and prognostic significance of TP53 in diffuse large B-cell lymphoma patients treated with R-CHOP: Report from an International DLBCL Rituximab-CHOP Consortium Program Study. Blood 2012; 120:3986–96.

34. Ameen R, Sajnani KP, Albassami A, Refaat S. Frequencies of non-Hodgkin's lymphoma subtypes in Kuwait: Comparisons between different ethnic groups. Ann Hematol 2010; 89:179–84.