DISCUSSION

Acute myeloid leukaemia is a heterogeneous clonal disorder of haemopoietic progenitor cells and the most common malignant myeloid disorder in adults. The median age at presentation for patients with AML is 70 years. Genetic defects are thought to be the most important factors in determining the response to chemotherapy and outcome.[53]

Current management of patients with AML is determined by a number of parameters, including age, performance status and the cytogenetic/molecular genetic characteristics of the leukemic clone. Together, these factors have an important bearing on treatment strategy, identifying potential candidates for molecularly targeted therapies and informing decisions on allogenic transplantation.[51]

Cytogenetic aberrations are among the most important independent prognostic factors in AML. A prognostic value of telomere shortening and telomerase activity has been suggested in various human hematopoietic malignancies including AML.[84]

It has been reported that increased hTERT and/or hTERC gene expression may be an important mechanism involved in unregulated telomerase activity. Accordingly, investigation of hTERT and/or hTERC amplification might be useful in the diagnosis and follow-up of cancer patients.[85]

The aim of the present study was to estimate human telomerase reverse transcriptase (hTERT) and human telomerase RNA component (hTERC) genes' copy number in adult AML patients using FISH and to correlate this with other clinical and laboratory parameters.

This study was carried out on a total of 25 adult patients (18-60 yrs) newly diagnosed with AML. The following results were obtained and analyzed.

In the present study, Interphase FISH analysis revealed hTERC gene amplification in 20/25 cases (80.5%). The percent of cells showing hTERC amplification ranged from 1% to 8% and the number of copies ranged from 3-6 copies. Htert gene amplification was found in 13/25 cases (52%). The percent of cells with hTERT amplification ranged from 1% to 7% and the number of copies ranged from 6-9 copies. Both genes were amplified in 52% of cases.

In accordance with our results, Eid et al., 2013[81], who carried out his study on 21 AML patients (16-67 yrs), found amplified hTREC and hTERT genes in 19/21 of AML patients (90.4%). The percentage of cells showing amplification, in their study, was much higher if compared to ours; 2% to 69% for hTERC and 3% to 76% for hTERT. They have demonstrated that telomerase can be a good cancer marker which may be involved in carcinogenesis of leukemia. Higher amplification was found in de novo cases than cases in remission which emphasize its role in clinical analysis, disease monitoring and detection MRD.

We and Eid et al.,2013[81] used the same probe and the same method of interpreting the results (amplification and no amplification), however we found the percentage of cells showing amplification lower in relation to them, the difference may be due to the fact that we followed the package insert obtained with the probes we purchased from Kreatech company, but this paper used another cut off value.

In the study conducted on hematological malignancies by Nowak et al., 2006[86], they found hTERC and hTRET genes' amplification in all samples tested (16 AML cases) and that leukemic cells possess variable number of hTERC and hTERT copies which ranged from 2 to 12 copies for hTERC and from 2 up to 60 for hTERT. They suggested that the activation of telomerase in leukemic cells is related to amplification of hTERT and hTERC genes and that the high expression and activity of telomerase found in leukemic cells may be partially explained by the amplification of hTERT and hTERC genes and that amplification of the telomerase genes seems to be a common event in carcinogenesis and may play a role in telomerase reactivation leading to cell immortalization. They showed that all leukemic cells tested have higher telomerase expression and activity, as compared to normal cells and it can be used to distinguish malignant from normal cells. They also conclude that high telomerase activity and expression in leukemic cells is not correlated with telomere length through detection of terminal restriction fragments (TRF).

These results were confirmed by Ying et al., 2008[63]. They proposed that increased TERT and/or TERC gene dosage is an important mechanism for up regulation of telomerase activity in human cancer on the basis of evidence that the levels of both TERT and TERC are limiting for telomerase activity and that the copy number of these genes is frequently increased in cancers by chromosomal gains or by amplification. They suggested that transduction of normal cells with hTERT expression constructs may result in up regulation of endogenous hTR expression, by mechanisms which are incompletely understood, they also suggested the possibility that increased copy number of genes encoding telomerase components has pro-oncogenic effects in addition to the ability of telomerase to synthesize telomeric repeats, prevent telomere shortening, and permit cells to escape from senescence and that detection of TERT and/or TERC amplification may have useful applications in cancer diagnosis and prognosis.

Also, Yirong et al., 2004[70] found elevated hTERT gene expression in 25/31 of newly diagnosed AML cases (80.6%) and in 20/24 (83.3%) of relapsed AML patients, and elevated hTERC gene expression in all 31 newly diagnosed AML cases (100%) and in all 24 relapsed cases, however they found hTERC gene expression not only in tumor cells but also in normal leukocytes, suggesting that hTERC may not play an important role during tumor-genesis. They demonstrated that most normal leukocytes contain very low telomerase activity and do not express hTERT and that the expression of hTERT was one of the important factors for occurrence of AML because there was significant difference between healthy volunteers and AML at initial presentation or at relapse. They came to a conclusion that up-regulation of telomerase activity and the expression of hTERT is correlated closely with the occurrence and relapse of AML and that hTERT and telomerase can be used to research occurrence mechanism of AML, observe curative effect, predict relapse of AML and estimate prognosis.

Also, Hee Jin Huh et al., 2005[87], who conducted his study on 21 AML Korean patients (13-74 yrs), found hTERT gene expression in 73% (8/11) of AML patients at diagnosis, 27% (8/29) of those from patients in CR, and 80% (8/10) of those in relapse. They demonstrated that hTERT mRNA expression was increased at diagnosis and during relapse in AML patients. They suggested that patients with high hTERT mRNA or with a trend toward increased hTERT mRNA tended to have a poor prognosis and disease

progression and that sequential and quantitative analysis of hTERT mRNA may be useful in predicting prognosis and monitoring disease progression in AML patients.

In another study conducted by Wang et al., 2010[88] on 79 AML Chinese patients (19-79 yrs) of them 49 were newly diagnosed, telomerase activity (TA) was found to be significantly higher in patients with AML compared with healthy individuals, average telomere length (TL) in AML patients (6.03 kb) was much shorter than in the control group (10.59 kb) and TL and TA were inversely correlated. They demonstrated that TA in M3 subtype was lower than non-M3 subtypes (including M2, M4, M5, M6 and M7). They suggested that the shortened TL and elevated TA in AML patients are mainly indicative of extensive proliferating activity and they correlate with disease progression and relapse. They concluded that patients in late-stage disease had shorter TL and higher TA than those in early stages thus the shortened TL and elevated TA are correlated with disease progression and relapse, and they may serve as prognostic factors for AML patients with poor outcome. M3 subtype is special with relative lower TA and long-lasting survival than other subtypes.

In contrast, Serakinci et al., 2002[89] found no amplification in either hTERT or hTERC genes in all samples tested (53 AML patients), arguing strongly against being a common phenomenon in AML. They suggested that hematological cancers differ from most other cancers in that they originate from cells with a low level of natural telomerase activity. Acquiring the necessary telomerase activity for cancer growth in leukemia thus only requires up-regulation of telomerase activity, whereas others cancers need to overcome a complete telomerase inhibition, possibly by amplifying telomerase genes to overwhelm telomerase repressors. So they came to a conclusion that telomerase up-regulation in hematological cancers and in solid tumor may develop along different pathways concluding that amplification is a property of solid tumors, not of hematological cancers.

Also Ozer et al., 2010 [90] found that none of the patients (23 AML adult Turks patients) (15-79 yrs) had hTERC gene amplification. They suggested that increased telomerase activity, via gene amplification, in the development of AML may not be as important factor as it is in solid tumors.

In our study, we could not found a significant correlation between hTERC and/or hTERT genes' amplification and other prognostic parameters (age, sex, organomegaly, PB findings, blast count in PB and BM , AML FAB type and remission after induction) ($p>0.05$).

In agreement with our results, Eid et al., 2013[81] could not establish a relation between amplification and other prognostic criteria except PB blasts but this study was conducted on different population; gulf Arabs.

Verstovsek et al., 2003[91] found no significant correlation between TA and age, platelet counts and hemoglobin level, however, TA was associated with total leucocyte counts. This may be attributed to a larger study group (65 AML patients).

Later, Hee Jin Huh et al., 2005[87] found no correlation between hTERT mRNA expression and sex, white blood cell counts, blast counts or complete remission; however, its expression was associated with the age of the patients. This difference may be due to that the study was conducted on a wider age range (13-74 years) and on different race; Korean.

Regarding AML FAB subtypes, Wang et al., 2010[88] found that TA in M3 subtype (n = 11) was significantly lower than non-M3 subtypes (M2, M4, M5, M6, M7) (n=38) this may be due to that Wang et al study was conducted on a larger number of patients than ours.

Regarding outcome, in our study we found a statistically significant correlation between hTERC and/or hTERT genes' amplification and the final outcome of patients; ($p=0.05$) for hTERC, ($p<0.001$) for hTERT.

Asfour et al., 2008[92] reported that high levels of telomerase activity in AML blasts predict unfavorable outcome, resistance to therapy and short survival.

Also, Wang et al., 2010[88] demonstrated that patients with a high TA level had a significantly poorer OS (31.65%); whereas, those with a low TA level had more favorable outcome (58.82%, $P < 0.01$) so they suggested that TA level can be considered as a reliable prognostic parameter in AML.

In contrast, Eid et al., 2013[81] found no statistically significant relationship between hTERC gene amplification and poor outcome patients than good outcome ones. However the hTERT gene amplification revealed a highly significant difference between good and bad outcome patients.

Regarding response to induction, in our study we could not find a statistically significant difference between hTERC and hTERT genes' amplification and achievement of CR.

Hee Jin Huh et al., 2005[87] found that patients with high hTERT mRNA expression had a low CR rate (33%) as compared to those without hTERT mRNA expression (80%). This may be attributed to the difference in ethnic groups; Korean.

The difference of our results in relation to results obtained by other groups may be attributed to difference in age group, number of the studied cases, different methodology and different studied populations.

SUMMARY

Acute myeloid leukemia is a heterogeneous clonal neoplasm characterized by accumulated genetic aberrations, which result in enhanced proliferation, block in differentiation and increased survival of the leukemic blast cells and is the most common malignant myeloid disorder in adults. It is characterized by an increase in the number of myeloid cells in the BM and an arrest in their maturation, frequently resulting in hematopoietic insufficiency with or without leukocytosis. It has an incidence of 2-3 per 100, 000 per year in children, rising to 15 per 100, 000 in older adults. It can occur at all ages but has its peak incidence in the seventh decade. Acute myeloid leukaemia accounts for approximately 25% of all leukemias in adults in the western world, and, therefore, is the most frequent form of leukemia.

Conventional methods for diagnosis of AML include peripheral blood and bone marrow examination, cytochemistry together with immunophenotyping by flowcytometry and conventional cytogenetics. Nowadays, molecular techniques are playing a major role in the diagnosis and identification of new abnormalities that may contribute to the susceptibility to AML. These molecular techniques include fluorescence insitu hybridization and polymerase chain reaction.

Cytogenetic analysis represents an important tool for both diagnosis and prognosis of AML. Among these cytogenetic data, the prognostic value of telomere shortening and telomerase activity has been suggested in various human hematopoietic malignancies.

Telomeres are repeated DNA sequences at the ends of chromosomes that are essential for chromosome protection and genomic stability. Telomerase is a ribonucleoprotein reverse transcriptase capable of compensating progressive telomere shortening. It is composed maily of two subunits: a catalytic subunit with reverse transcriptase activity (TERT), an RNA component (TERC) that acts as a template for DNA synthesis. These two subunits are encoded by two different genes located on 5p15 and 3q26 respectively, both are essential for the function of the enzyme. Telomerase is necessary for the long-term proliferation potential of human stem cells and cancer cells, and for normal tissue renewal. Ectopic expression of telomerase in normal human cells leads to extension of life-span or immortalization of many cell types.

In most cancers including AML, telomerase is expressed at levels that are substantially higher than in normal cells. A known consequence of telomerase up regulation (which is considered to play a critical role in oncogenesis) is maintenance of telomere length, and thus evasion by cancer cells of apoptosis that are associated with the steady decrease in telomere length in normal cells.

The aim of this study was to estimate human telomerase reverse transcriptase (hTERT) and human telomerase RNA component (hTERC) genes' copy number in adult AML patients using fluorescence in situ hybridization (FISH) and to correlate this with other clinical and laboratory parameters.

This study was carried out on 25 adult patients (18-60 yrs) newly diagnosed with AML. All patients were subjected to full history taking, complete clinical examination and investigations. The investigations included CBC, bone marrow aspiration,

immunophenotyping and estimation of human telomerase reverse transcriptase (hTERT) and human telomerase RNA component (hTERC) genes' copy number using FISH.

In the present study, we found hTERC gene amplification in 20/25 cases (80.5%) and hTERT gene amplification in 13/25 cases (52%).

No statistically significant differences were found between hTERC and hTERT genes' amplification and other prognostic parameters. However, regarding outcome, we found a statistically significant difference between hTERC and/or hTERT genes' amplification and the final outcome of patients.

From the results of our study, we concluded that hTERC & hTERT genes are frequently amplified in AML patients and that although we could not find a signifcant relation of hTERC and hTERT genes' ampilification with known prognostic factors, we found a significant relation between hTERC & hTERT genes' ampilification with the final outcome of patients so they can be used for following disease progression & predecting the outcome of patients.

CONCLUSIONS

- hTERC and hTERT genes are frequently amplified in AML patients therefore telomerase can be a good cancer marker which may be involved in carcinogenesis of leukemia.

- Although other groups found a significant relation, we could not find a signifcant relation of hTERC and hTERT genes amplification with known prognostic factors (age, sex, organomegaly, PB findings, blast count in PB and BM , AML FAB type and remission after induction)

- However, there is a significant relation between hTERC and/or hTERT genes amplification with the final outcome of patients so they can be used for following disease progression and predicting outcome.