Clinical Significance of Serum p53 and Epidermal Growth Factor Receptor in Patients with Acute Leukemia

Mohamed Mohamed Abdel-Aziz

Abstract

Background: Pretreatment serum p53 and epidermal growth factor receptor (EGFR) were assessed using enzyme-linked immunosorbent assay (ELISA) in patients with acute leukemia to analysis their roles in characterization of different subtypes of the disease. Materials and Methods: Serum samples from thirty two patients with acute myeloid leukemia (AML) and fourteen patients with acute lymphoid leukemia (ALL) were analysed, along with 24 from healthy individuals used as a control group. Results: The results demonstrated a significant increase of serum p53 and EGFR in patients with AML (p<0.0001) compared to the control group. Also, the results showed a significant increase of both markers in patients with ALL (p<0.05, p<0.0001 respectively). Sensitivities and specificities for these variables were 52% and 100% for p53, and 73.9%, 95.8% for EGFR. Serum p53 and EGFR could successfully differentiate between M4 and other AML subtypes, while these variables failed to discriminate among ALL subtypes. A positive significant correlation was noted between p53 and EGFR. Negative significant correlations were observed between these variables and both of hemoglobin (Hg) content and RBC count. Conclusions: Mutant p53 and EGFR are helpful serological markers for diagnosis of patients with AML or ALL and can aid in characterization of disease. Moreover, these markers may reflect carcinogenesis mechanisms.

Keywords: Acute myeloid leukemia - acute lymphoid leukemia - p53 - EGFR - ELISA - ROC
cells are. This was based largely on how the leukemia cells looked under the microscope after routine staining. The subtypes of AML involved in this study were M1 (26%), M2 (19.5%), M3 (15.2%), and M4 (8.7%), while the subtypes of ALL were ALL1 (15.2%) and ALL2 (15.2%). The control group included 24 healthy individuals (18 men and 6 women; mean age 33 years, range 24-42). The control individuals were selected without a clinical history of any chronic diseases and without symptoms or signs of acute or chronic leukemia. Peripheral blood samples were obtained from the patients and those from healthy subjects in the control group and sera were promptly separated and stored at −20°C till use. The study was approved by the local Research and Ethics Committee of Mansoura and Cairo Universities. An informed consent was obtained from the child’s parent or guardian before inclusion in the study.

**Analysis of hematological data**

Peripheral blood samples were obtained from all studied groups (healthy individuals, acute myeloid leukemia, and acute lymphoid leukemia) to analyze the hematological parameters as hemoglobin content (Hb), red blood cells (RBCs), white blood cells (WBCs), and platelets count according to routinely investigated laboratory tests.

**Serum p53 and EGFR analysis using ELISA**

A home-ELISA method was optimized to obtain the optimum reaction conditions. Polystyrene microtiter plates were coated with 50 µl/well of each serum sample diluted 1:1000 in carbonate/bicarbonate buffer (pH 9.6). The plates were incubated overnight at room temperature and washed three times using 0.05% (v/v) PBS-T20, was added and incubated at 37°C for 2 h. After washing, 50 µl/well of anti-mouse IgG alkaline phosphatase conjugate (Sigma), diluted 1:250 in PBS-T20, was added and incubated at 37°C for 1 h. Excess conjugate was removed by extensive washing and the amount of coupled conjugate was determined by incubation with 50 µl/well p-nitrophenyl phosphate (Sigma) for 30 min at 37°C. The reaction was stopped using 25 µl/well of 3M NaOH and absorbance was read at 405 nm using microplate autoreader (Bio-Tek Instruments, WI, USA). Cut-off level of ELISA above or below which the tested samples were considered positive or negative was calculated as the mean concentrations of 24 serum samples from healthy individuals ±2SD.

**Statistical analysis**

Results were expressed as mean±SD, and were analyzed by using X²-test, Mann–Whitney U-test, Fisher’s exact test, Spearman correlation as appropriate. The Mann–Whitney U-test was used to compare different groups for continuous variables including the serum levels of mutant p53 and EGFR. The correlations between serum levels of mutant p53, EGFR, and hematological data of patients were assessed by Spearman correlation. p<0.05 was considered significant. These statistical procedures were performed using SPSS software, version 11 for windows (SPSS Inc., USA). Receiver operating characteristic curves (ROCs), area under curve (AUC) calculations, and one-way analysis of variance (ANOVA) to compare among different subtypes of patients with acute leukemia were performed using MedCalc software, version 12 for windows (Belgium).

**Results**

**Hematological data of the studied groups**

The hematological data of all studied groups (HI, AML, and ALL) were listed in Table 1. Hg content, RBCs, and platelets count of patients with AML or ALL were significantly lower (p<0.0001 for all except platelets count in ALL group, p<0.05) than those in healthy individuals, while WBCs count was significantly higher (p<0.0001 for both AML and ALL groups) than those in healthy individuals.

**Serum levels of p53 and EGFR**

As shown in Table 2, the results demonstrated a significant difference between the serum levels of mutant p53 and EGFR in patients with AML compared to that of controls (p<0.0001 for both p53 and EGFR). Also, serum EGFR and p53 levels were increased significantly in patients with ALL compared to the control group (p<0.0001 for EGFR, p<0.05 for p53). Our results showed that the positivities of p53 and EGFR in patients with AML were 60% and 78.12% respectively, while the positivities in patients with ALL compared to the control group were 61.53%. It is observed from our results that the serum level of p53 in patients with ALL was significantly lower (p<0.01) than those in patients with AML, but there was

| Table 1. Age, Sex, and Hematological Data of Patients with Acute Leukemia |
|-----------------------------|------|-------------------|------|-------------------|------|-------------------|
| Variable                  | HI Mean±SD | Range | p value | AML Mean±SD | Range | p value | ALL Mean±SD | Range | p value |
| Sex (M/F)                  | 18/6 | 24-42 | 19/13  |   |   |   | 9/5 | 27.38±7.27 | 18-41 | NS |
| Age (y)                    | 33±5.3 | 11.7-14.0 | 32.61±10.95 | 14-53 | NS |   | 6.97±2.31 | 2.7-12.9 | <0.0001* |   |   |
| Hb (g/dl)                  | 12.52±0.78 | 4.62±0.44 | 3.8-5.2 | 6.42±0.44 | 3.8-5.2 | 6.3-7.1 | 4.62±0.44 | 3.8-5.2 | <0.0001* |   |   |
| RBCsX10^6/ml               | 6.4±1.22 | 41.14±4.27 | 3.7-8.1 | 41.14±4.27 | 3.7-8.1 | 3.7-8.1 | 209±25 | 175-250 | <0.0001* |   |   |
| WBCCX10^6/ml               | 52.1±60.18 | 47.7±47.73 | 1-180 | 47.7±47.73 | 1-180 | 1-180 | 209±25 | 175-250 | <0.0001* |   |   |
| PlateletsX10^9/ml          | 48.71±47.73 | 3-165 | 3-165 | 3-165 | 3-165 | 3-165 | 209±25 | 175-250 | <0.0001* |   |   |

*HI, healthy individuals; AML, acute myeloid leukemia; ALL, acute lymphoid leukemia*
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Table 2. Serum p53 and EGFR Levels in Patients with Acute Leukemia

<table>
<thead>
<tr>
<th>Study group</th>
<th>p53 (ng/ml)</th>
<th>EGFR (Optical density)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positivity (%) Mean±SD</td>
<td>p value</td>
</tr>
<tr>
<td>Healthy individuals (HI)</td>
<td>0</td>
<td>0.14±0.022</td>
</tr>
<tr>
<td>Acute leukemia (total patients) (AL)</td>
<td>52</td>
<td>0.21±0.08 &lt;0.0001&quot;</td>
</tr>
<tr>
<td>Acute myeloid leukemia (AML)</td>
<td>60</td>
<td>0.22±0.087 &lt;0.0001&quot;</td>
</tr>
<tr>
<td>Acute lymphoid leukemia (ALL)</td>
<td>8.69</td>
<td>0.18±0.07 &lt;0.05&quot;</td>
</tr>
</tbody>
</table>

*Asterisks denote that p value is significant. NS, not significant

Table 3. Correlation Amongst Serum p53, EGFR and Hematological Data

<table>
<thead>
<tr>
<th>Variable</th>
<th>p53 Spearman’s correlation (p)</th>
<th>EGFR Spearman’s correlation (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR</td>
<td>0.85 (&lt;0.0001*)</td>
<td>-</td>
</tr>
<tr>
<td>Hb</td>
<td>-0.57 (&lt;0.0001*)</td>
<td>-0.42 (&lt;0.01*)</td>
</tr>
<tr>
<td>RBCs</td>
<td>-0.5 (&lt;0.001*)</td>
<td>-0.43 (&lt;0.01*)</td>
</tr>
<tr>
<td>WBCs</td>
<td>0.03 (NS)</td>
<td>0.14 (NS)</td>
</tr>
<tr>
<td>Platelet</td>
<td>-0.07 (NS)</td>
<td>-0.08 (NS)</td>
</tr>
</tbody>
</table>

*Asterisks denote that p value is significant. NS, not significant

Figure 1. Receiver Operating Characteristic Curve (ROC). A) p53 Protein. The Area Under Curve (AUC) is 0.8 and B) Epidermal Growth Factor Receptor. The Area Under Curve (AUC) is 0.93

Figure 2. Box Plot Comparing Mean Serum Levels. A) p53 and B) EGFR in Different Subtypes of Patients with Acute Leukemia (L1, L2, M1, M2, M3, and M4) Using One-Way Analysis of Variance (ANOVA)

Discussion

p53 biosignatures contain useful information for cancer evaluation and prognostication (Anensen et al., 2012). In the present work, there were significant increase in the serum levels of p53 in patients with AL, AML (p<0.0001, positivity 52%, 60% respectively), and ALL (p<0.05, positivity 8.69%) compared to control group. Also, the serum p53 levels in patients with AML were higher than those in ALL group with a statistically significant difference (p<0.01). These results indicate that, the expression of p53 protein may have a different mechanism in the pathogenesis progress in these two types of acute leukemia and it may be considered as a helpful marker to differentiate between them. Several studies revealed that the mutation of p53 gene has been reported only 5-10% of patients with AML (Diccianni et al., 1994; Hsiao et al., 1994; Wattel et al., 1994; Zhu et al., 1999). In contrast, the results of Sahu and Jena (2011) showed that 91% patients with AML were p53 immunopositive using...
immunocytochemistry. Also, measurement of p53 protein expression by flow cytometry showed higher percentage of p53 expression in cells of AML patients at the time of diagnosis opposite to the controls (Konikova et al., 1999). Furthermore, the results of Park et al. (2000) revealed that the overexpression of p53 protein was found in 38% of patients with AML, while 25% of patients with ALL were p53 immunopositive using immunohistochemical technique. Significant increase of serum p53 protein in different human cancers were reported by several authors (Segawa et al., 1997; Suwa et al., 1997; Shim et al., 1998; Sobti and Parashar, 1998; Morita et al., 2000; Charuruku et al., 2001; Chow et al., 2001). In addition, our previous reports revealed an increasing level of serum p53 protein using ELISA in different gastrointestinal tumors (Abdel-aziz et al., 2005), hepatocellular carcinoma (Abdel-aziz et al., 2005), and colorectal cancer (Abdel-aziz et al., 2009).

Epidermal growth factor (EGF) and its receptor (EGFR) are one of the most important ligands/receptors of mammalian cells. EGFR possesses intrinsic tyrosine kinase activity, and its overexpression is associated with malignant transformations (Schlessinger and Ullrich, 1992; Rajkumar, 2001). Previous studies reported that EGF/EGFR binding plays an important role in the carcinogenesis of several human tumors because EGF stimulates proliferation of malignant cells through its receptor, EGFR (Yamazaki et al., 1998). In the present study, there was a significant increase in the EGFR level in both of AL and AML patients groups compared to the control group (p<0.0001, positivity 73.91% and 78.12% respectively). Furthermore, EGFR levels in ALL patients were significantly increased (p<0.0001) with a high positivity (61.5%) compared to control group, while there was a not quit significant difference in EGFR levels between AML and ALL patients groups.

The present work showed that the optimized home – ELISA technique allows the serological quantitative analysis of these markers (p53 and EGFR) to give different sensitivities for each with good specificities (for p53, 52%, 100%, for EGFR, 73.91%, 95.8%). AUC for each marker was calculated according to their ROCs and it was found that the AUC for p53 and EGFR are 0.8, and 0.93 respectively. These results indicate the good validity for p53 and EGFR to discriminate the seropositive from the seronegative samples of AL patients and indicate that our optimized ELISA method is a reliable diagnostic technique for differentiation between positive and negative cases.

Furthermore, our results showed significant positive correlation between p53 and EGFR. Thus, it seems clear that these markers are dependent variables. On the other hand, there were significant negative correlations between these variables and some hematological data of AL patients as hemoglobin content and red blood cells count, while there were no correlations with white blood cells and platelets count.

The most important rationale of this study is to analyze the serum levels of the these variables in different subtypes of both AML and ALL patients. Our results showed that both of serum p53 and EGFR levels in M4 subtype are higher than those in M1, M2, and M3 (for p53, p=0.03, for EGFR, p=0.028), while there were no significant differences among the subtypes M1, M2, and M3. These results showed that the serological analysis of these markers have a significant role for characterization of AML subtype. In contrast, serum levels of these markers failed to discriminate between the two subtypes of ALL (ALL1 vs ALL2).

In conclusion, our optimized ELISA technique is a valid reliable assay for determination of serum p53 and EGFR and these markers are helpful serological markers for diagnosis of both AML and ALL patients and can discriminate between different types of AL patients. Furthermore, these variables can differentiate among the different subtypes of AML patients and aid for disease characterization. Our results encourage us and others to investigate the efficacy of these markers to monitor patients with AL during and after treatment.

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References


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