



**SDI Review Form 1.6**

Journal Name:	<a href="#">Journal of Pharmaceutical Research International</a>
Manuscript Number:	<b>Ms_JPRI_36743</b>
Title of the Manuscript:	<b>The expression patterns of APC2 and APC7 in newly diagnosed acute lymphoblastic leukemia</b>
Type of the Article	<b>Original research paper</b>

**General guideline for Peer Review process:**

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound. To know the complete guideline for Peer Review process, reviewers are requested to visit this link:

(<http://www.sciencedomain.org/page.php?id=sdi-general-editorial-policy#Peer-Review-Guideline>)



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**PART 1: Review Comments**

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
<b>Compulsory</b> REVISION comments	<p>Major concern about the calculation of the fold induction of gene expression. The authors did not use the <math>2^{-\Delta\Delta Ct}</math> method, instead they use the following formula: "<math>\Delta Ct</math> was calculated from <math>C_{T, target\ genes} - C_{T, ABL}</math> formula and <math>2^{-\Delta Ct, case} / 2^{-\Delta Ct, control}</math> was considered as gene expression fold changes" to calculate gene fold induction. The deduction of this formula was not described or mathematically proven by the authors or at least a reference should be added. The results should be re-calculated using the <math>2^{-\Delta\Delta Ct}</math> method, where <math>\Delta Ct</math> refers to the normalization of all genes and samples studied to the housekeeping gene and <math>\Delta\Delta Ct</math> is the difference between <math>\Delta Ct</math> values of ALL patients vs <math>\Delta Ct</math> values of control samples.</p> <p>Statistical analysis – The number of independent experiments was not disclosed in the material and methods or results sections, or even in the figure legends. The data presented should be the result of at least three independent experiments to have any statistical value/significance. The P values should be calculated from the averages of each independent experiment (for instance if 3 independent experiments were done, N=3) and the number of replicas should not be included in the calculation of P values.</p> <p>Figure legends are extremely incomplete, they only contain the figure title and lack methodological detail, statistical information and explanation of data analysis.</p> <p>Ethical issue: they have been addressed by the authors. The work involves the collection of clinical samples, these were done with patients consent according to the Helsinki's Declaration.</p>	<p>Thomas D Schmittgen &amp; Kenneth J Livak in 2008 published an article entitled as "Analyzing real-time PCR data by the comparative CT method". In this article, the forth example is about gene expression analysis in case-control studies or in studies that samples are collected from different tissues. In this part authors mentioned, <math>2^{-Ct, case} / 2^{-\Delta Ct, control}</math>, control must considere as relative gene expression (fold change).</p> <p>Due to our work foundation limitation, we could not do other techniques to confirme qPCR data. The reported P values were calculated from triplicate qPCR expriments and gathered data analysed with statistical test that are mentioned in the related parts.</p> <p>This work done in agreement with ethnic committee of Shahid Beheshti university of medical sciences. In the consent form and for collection of all samples we include all ethnical aspects of Helsinki's Declaration</p>
<b>Minor</b> REVISION comments	<p>English grammar errors throughout the manuscript that need to be corrected. Table 2 – some of the values between brackets are missing the % symbol</p>	We revised the paper according to your comments.
<b>Optional/General</b> comments	<p>Standard deviations are quite significant particularly in the control samples which is a concern.</p> <p>Overall I feel that there are substantial issues with data analysis of the RT-qPCR data and I am unclear of the number of independent experiments done, I also think that the manuscript does not have enough data to be published. For this reason I would like to suggest additional experiments that would strengthen the manuscript: Knocking down or knockout the APC2 and APC7 genes in ALL cell lines should bring some more information to the role of these proteins in ALL, for instance by comparing cell proliferation/ apoptosis of KD versus WT cell lines. The manuscript would also greatly benefit from protein analysis – protein extraction followed by western blotting of clinical samples – to support the gene expression results.</p>	<p>Although it seems that standard deviation significant but it was statically acceptable. we used Livak recommendations for RQ-PCR data analysis. The experiments were done in triplicates. Unfortunately it is not possible to do suggested extra experiments due to our work foundation limitations.</p>