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Quantification of DNA damage by the analysis of silver stained comet assay images

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Abstract

1. Objectives

The main objective is to develop a fully automated system for Deoxyribo Nucleic Acid (DNA) damage analysis using silver stained comet assay images for clinical applications.

2. Materials and methods

Silver stained comet assay images of lymphocytes obtained from confirmed cases of Schizophrenia were employed for DNA damage analysis using Single Cell Gel Electrophoresis (SCGE) or Comet assay. The image analysis consists of three stages: (1) Comet segmentation by incorporating shading correction, pre-processing stages for image enhancement, noise filtering and thresholding; (2) Comet partitioning by fuzzy clustering and (3) Comet quantification stage in which the limitations of fuzzy clustering are eliminated.

3. Results

This study involves 40 cases of Schizophrenia. Results of the proposed method are compared with those of OpenComet and CometScore software. Comet segmentation result is validated with a confusion matrix which shows better values for all performance indices. The proposed technique gave 87.12% Positive Predictive Value (PPV) and 90.16% sensitivity. It shows a large improvement in PPV and sensitivity, over the most recent competitive method, OpenComet. Regression analysis is carried out to validate the quantification results. Visual scores evaluated by the expert and DNA (%) in tail are used for finding the coefficient of determination, r^2 statistic. The proposed method gives a better r^2 value of 0.9. The performance of the proposed method is evaluated by a clinical expert.

4. Conclusion

The proposed method is found to be very efficient for DNA damage analysis of silver stained comet assay images in the area of clinical research.

Keywords: DNA damage analysis; Silver stained comet assay images; Comet segmentation; Comet partitioning; Fuzzy clustering

1. Introduction

The analysis of DNA damage is carried out in many fields of medicine such as toxicology, pharmacogenomics, oncology, human epidemiology and biomonitoring. DNA damage occurs due to continuous exposure of cells to exogenous and endogenous agents. The DNA breaks thus formed can block DNA replication and transcription, and if they are not repaired or repaired incorrectly, it may lead to Alzheimer's disease, Parkinson's disease, cancer, premature aging, diabetes mellitus, mental illness, heart diseases etc. DNA damage analysis provides valuable information regarding early biological effects of hazardous chemicals and important information about the different stages of diseases. This information could be used by clinicians and pathologists for planning the treatment and determining the

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best course of intervention. Hence the analysis of DNA damage has great significance in clinical research and there is a clear demand for an accurate, fast and sensitive method to detect DNA damage [1].

Comet assay is a widely accepted method for assessing DNA damage in individual cells. It can be used to measure single-strand or double-strand DNA breaks, alkali labile sites, DNA crosslinks, base/base-pair damages and apoptotic cells. This method was introduced by Ostling and Johanson [2] in 1984, in which they used neutral conditions to measure DNA single strand breaks. Subsequently, in 1988 a modified version was introduced by Singh et al. [3], which used alkaline conditions that improved its reproducibility and specificity.

In comet assay, during electrophoresis the negatively charged fragments of DNA migrate out from the nucleus, move towards the anode and form a comet like structure, and hence the name comet assay. Such a comet can have head and tail regions [4]. Depending upon the level of DNA damage the comet will have different sizes and shapes. The DNA damage can be quantified by measuring percentage DNA in tail, tail length and tail moment [5,6]. Even-though the tail moment is considered as the most important parameter for DNA damage analysis, the percentage DNA in tail is preferred, as it is both informative and very easy to interpret [4]. The percentage DNA in tail is determined by comet scoring. The comets are scored in three different ways: (1) Manual method, (2) Semi automated method and (3) Fully automated method. In semi automated and automated methods, image analysis is based on computer programs.

Manual methods [7] require skilled operators. But still visual scoring is usually used as a benchmark to validate the results obtained through automated algorithms. Semi automated methods were developed by Rivest et al. [10], Helma and Uhl [8] and Konca et al. [9]. CASP [8,9] and CometScore are two semi automated open source software packages available in the internet. The disadvantages of semi automated software packages are their low throughput and lack of reproducibility of results. Over and above, the requirement of experts in different stages makes the technique user dependent, tedious and time consuming process.

Fully automated systems were developed by Bocker et al. [13], Frieauff et al. [14], Dehon et al. [15,16], Sansone et al. [17], Gonzalez et al. [18] and Gyori et al. [12]. Most of these methods were developed for fluorescent stained images except the methods proposed by Gonzalez et al. and Gyori et al. Comet assay image analysis based on fluorescent images requires high quality fluorescence microscope and the cells should be photographed and analysed immediately as the slides cannot be stored for a long period.

The current work on DNA damage analysis is carried out using silver stained comet assay images since they are preferred in clinical applications. The advantages of silver staining method are the following: it is inexpensive, slides can be preserved for a long time, the straining procedure is less hazardous than fluorescent staining and the analysis can be carried out using a simple light microscope, which is available in almost all laboratories. However, the background noise of silver stained images is higher than that of fluorescent stained images. Therefore, detection of true comets from the very noisy silver stained images is a challenging task. Most of the available tools for comet assay image analysis for silver stained images are of semi automated type. They are operator dependent and time consuming because scoring of at least 50 cells per case is required. Thus, there is a strong need to develop a fully automated system with superior performance in detecting true comets and quantifying DNA damage from highly noisy silver stained comet assay images.

CellProfiler and OpenComet are two open source software available in internet to analyse silver stained comet assay images. With CellProfiler [19], Gonzalez et al. [18] modified the pipeline provided in the website by adding a background subtraction module and two filtering modules to quantify DNA damage. The performance of the CellProfiler software was evaluated with all the 40 cases under study and the TP (%) (True Positive) obtained was only 46.15%.

Gyori et al. [12] developed OpenComet for the analysis of comet assay images. It is developed as a plug-in for the image processing platform, ImageJ. In OpenComet, comet segmentation is based on geometric shape attributes and comet partitioning is based on image intensity profile analysis. This tool can be used for the analysis of both fluorescent and silver stained images.

This paper specifically focuses on addressing the difficulties in analysing noisy silver stained comet assay images. In the proposed automated method, comet segmentation is carried out by using a pre-processing stage along with morphological and thresholding operations. Fuzzy based clustering algorithm is used for comet partitioning and finally comet parameters are quantified effectively after rectifying the limitations of fuzzy clustering.

2. Materials and methods

The method of obtaining silver stained images starts with sample collection and lymphocyte separation followed by comet assay procedure. The comet assay images are obtained at the final stage of comet assay procedure, which are used to quantify DNA damage.

2.1. Sample collection

After obtaining written and informed consent, blood samples were drawn from peripheral vein of confirmed cases of Schizophrenia in the age group of 18 to 65 years admitted in Psychiatry ward of a tertiary care hospital in South India, and lymphocytes were separated by centrifugation. Patients with Schizophrenia have greater DNA damage than the normal population due to various mechanisms involving the redox status in these patients [20,21] and hence were chosen as cases in the study. The sample size is 40 patients.

2.2. Comet assay procedure

The first step in comet assay procedure is the preparation of homogeneous slides to keep the noise level as low as possible.