Automated Histopathological Diagnosis of Pediatric Medulloblastoma – A Review Study

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Abstract

Microscopic image based analysis plays an important role in histopathological computer based diagnostics. Identification of childhood medulloblastoma and its proper subtype from biopsy tissue specimen of childhood tumor is an integral part for prognosis. The extent of survival of patients highly depends on prior identification and treatment of childhood Medulloblastoma. Computer based algorithms and techniques have been used to assist pathologist and doctors by means of various methodologies. Clinical diagnosis was studied to gain insight of the malignancy and the phases a patient undergoes when diagnosed with childhood Medulloblastoma. This paper presents an overview of the work in the area of childhood Medulloblastoma by image processing, addressing several issues in histopathological processing including slide preparation, microscopic observation, clinical features, diagnostic evaluation, texture analysis, feature extraction and classification. It is a very recent topic of importance and seriousness as it is a pediatric CNS tumor. This review will present an insight to readers about the clinical aspects and computer based trials by scientist for Computer aided diagnostics of childhood Medulloblastoma.

Keywords: Medulloblastoma, image processing, histology, H&E stain

INTRODUCTION

Medulloblastoma (MB) is the most common embryonal central nervous system (CNS) tumor of childhood [1] and is comprised of biologically different subsets of tumors arising from stem and/or progenitor cells of the cerebellum. It begins in cells in the cerebellum and is defined as a densely cellular, cerebellar tumor that arises over the roof of the fourth ventricle (Fig 1) [2]. Although it accounts for 6%–8% of all central nervous system tumours, presence of such tumors are commonly found in the pediatric age group [3–4]. Graham and Lantos [5] reported it as “a malignant invasive embryonal tumor of the cerebellum with prefental manifestation in children and adolescents and an inherent tendency to metastasize via CSF pathways”. The CNS which is made up of the brain and spinal cord, controls all vital functions of a human body. This includes thought speech and body strength. An improved 2- and 5-yr survival rate with correct diagnosis has been reported for childhood MB [6]. Apart from having a very poor survival history if not diagnosed and treated in time, it has severe side effects, as the cerebellum controls body movement and coordination. According to WHO classification of CNS tumors [7] four distinct histopathological subtypes of MB have been described: classic, desmoplasic, MB with extensive nodularity and large cell/anaplastic MB. Microscopic view of the tissue level has sheets like the arrangement of closely packed cells, with large dark size nuclei. This classic pattern is mostly prevalent. As reported by Chabbda et. al. [8] the delivery of an integrated diagnosis is of great clinical value as both the histological and molecular variants have distinct therapeutic and prognostic implications. The diagnosis of pathologists is more likely affected by inter and subjective variations of pathologist’s knowledge and experience and hence universal assessments of cases are very necessary for a productive objective. Computer aided technique for histological image analysis has played a major role in the recent pastyears, and research is being made in the field for more precise outcome. According to Polednak and Flannery [9] MB constitute approximately 20% of all childhood primary nervous system tumors and its annual incidence in the United States is estimated at 0.2/100,000 for whites and 0.1/100,000 for blacks. A retrospective study [10] of CNS tumors done over a period from 1996 to 2012 in the region, in the Dept. of Pathology of Guwahati Neurological Research Centre, Sixmile, situated in the region where this study was conducted, reported that out of a total of 980 CNS tumors 20 were MB, a majority (90%) of which occurred in
posterior fossa. The CNS tumors were studied and tabulated according to histologic type, age, groups, sex, and site. It was observed in the study that MB tumours constitute 2.04% of all CNS tumors in the series, a finding similar to other studies across the world. Moreover the percentage occurrence age group-wise were found to be 40% (below 10 years) and 70% (0-20 years). The study completed at GMCH reported the distribution of the disease with respect to age, sex, clinical signs and histological variants. 42 (64.6%) belong to 5-15 years and constituted the most common age group. 11 cases (16.9%) belong to 3-5 years group, 9 cases (13.8%) in 0-3 years and 3 cases (4.6%) above 15 years. Of the sample 40 (61.5%) were male and 25 (38.4%) were female.

**MOTIVATION**

The present review is to make a complete analysis of the computer aided diagnostic tools developed or reported for analysis of this particular type of tumour. MB is not a cancer area which may call for early detection. All patients who are diagnosed with presence of a tumour through a CT or MRI have to be operated. Complete resection of the tumour is the surgical gold standard for MB as it is associated with better prognosis. Moreover medulloblastoma manifests itself pretty rapidly, as it is a rapid growing tumor. Hence, it would be further helpful if once categorized as MB, it could be further categorized into its various subtypes. Although innumerable computer oriented image processing and pattern recognition methods have been developed and reported in the field of medical diagnostics it is well known that the cell and tissue structure varies from site to site. Hence it is pertinent to find out that method which is most suitable for application in the area of concern. For lack of such a method it then becomes necessary to develop a novel method, giving the best results.

**FEATURE DESCRIPTION RELEVANT TO OUR STUDY**

**Macroscopic Appearance**

This tumour appears well defined, i.e. spread-out rather than an accumulated lesion. When cut the surface is found to be finely granular of grayish pink color and of softly consistent. There may be occasional haemorages but mostly without macroscopic necrosis [5]. Approximately 25% of the tumour is found to originate from the granule neuron precursor cells(GNPC’s) after unusual activation of the sonic hedgehog pathways (SHH). Evidence suggest subtypes of MB have distinct developmental origins [11].

**Microscopic appearance**

Within the tumour the childhood MB cells are densely packed with a round to oval or carrot liked shape and scanty cytoplasm. Sometimes a nodular pattern is observed with interspersed septae [5]. Fig 2 reveals how the microscopic appearance of each individual subtype differs. The classic pattern has sheets like arrangement of the cells with extensive cellularity (b), the large cell has same arrangement like the classic subtype but the size of the cells are enlarged but unlike classic is highly aggressive in nature. Large cell MB is a highly malignant variant with cells that have large, round or pleomorphic, moulded nuclei with nucleoli and more abundant cytoplasm than non-large cell MB. Here, cell-cell wrapping, large areas of necrosis, high mitotic activity and high apoptotic rate are common [12]. (c), The nodular pattern has nodules like structure in the distribution of cell arrangement (a) while the last subtype of desmoplastic have network of collagen fiber (d) [5]. Desmoplastic and nodular MB are characterized by Reticulin-free nodules of tumor cells, with neuronal or astrocytic diversity divided by dense strands of an intercellular network of reticulin fibers. The nodules could be hypocellular or hypercellular relative to surrounding MB and frequently contain the same fine fibrillar stroma present in the surrounding MB [13]. Of all types of MB, Desmoplastic type has the highest rate of improved survival. In the WHO 4th edition, Nodular is defined histologically as “

![Figure 2: The architectural difference of each subtype](image)

To identify the four different subtype of MB, tissue specimens are obtained by state medical centers through proper procedures of surgery in GMCH, Neurological Department. The surgical management procedure is detailed below:

1. **CSF Diversion Procedure before Definitive Procedure:**
   Indication: Patients report with signs and symptoms of raised intra cranial pressure with decreased consciousness level (not improved by pre-operative dexamethasone and Mannitol administration) and CT scan showing hydrocephalus.

2. **Definitive Procedure:** The patients undergo sub-occipital craniotomy and excision of the lesion. The extent of resection is determined intra-operatively (by surgeon’s opinion) and by
post-operative MRI of brain. Depending upon the extent of excision, the excision is classified in four categories:

- Gross Total: When no residual tumour tissue left (on the basis of intraoperative surgeon’s opinion and on post op Gadolinium enhanced MRI).
- Near Total: When more than 90% of tumour tissue is resected.
- Subtotal: When less than 90% of tumour tissue is resected.
- Biopsy Only: When less than 10% of tumour mass is resected.

**Histopathology**

It involves study of tissue samples under microscope to examine cells and disease in fine detailing. The preparation for tissues under microscope involves preparation of slides. For the preparation of the slides, the tissue blocks are sectioned and placed on glass slides where they are stained using Harri’s Haematoxylin and Eosin solution which results in a color separation of the different components of the tissue sample. A well-stained slide would result in the nuclei as blue, Acidophilic nuclei as red, Basophilic nuclei as blue, Muscle, Collagen and Fibrils as pink, Eosinophilic granules as red and basophilic granules as blue. Johansson et al. [14] described how histological slides are prepared.

**Computer Aided Diagnosis**

Computer aided diagnosis tools are being used since decades as a medical diagnosis tool. It can be tracked back to 1990’s with the introduction of digital mammographic tool [15]. Researchers from both technical (computer image analysis) field and clinical (pathologist) field value the introduction of CAD based system for quantitative analysis. Digital analysis of histological samples gives a whole wealth of information rather than only classifying into benign and malignant. Researchers across the globe is using CAD based methodologies not to replace but make an additional benefit for pathologist. Waheed et al. [16] has used CAD based system for classification of renal cell carcinoma. Kather et al. [17] has done a multiclass textural classification for colorectal cancer histology. Kumar et al. [18] has used CAD for detection of cancer from microscopic biopsy images. Therefore CAD based methods can also be a tool for classification of childhood MB biopsy samples.

**METHODS**

**Literature search**

A search on different database with keywords “histological image processing”, “MB histological image processing” and “MB image processing histological slide” was performed by us to have a knowledge of the past studies on histological image processing along with the methodologies used both at cellular and architectural level and to have an idea of the relevant work on MB histological image analysis. Papers from IEEE Xplore, Scientific Reports, Elsevier, Pubmed, etc. which were relevant to our topic were downloaded and studied.

**Paper selection**

We considered for our study those papers that contained works related to cytological cell image classification or digital histological analysis and also works on MB image analysis. We surveyed a total of 1493 papers from different repositories out of which we found 58 of our interest. MB image processing is a very recent topic, and out of the 58 papers, we found only four papers on MB histological image processing in recent years. The papers that were not considered for our interest consisted of immunohistochemistry, animal organ study, a study relating to the chemical composition of the histological slide, brain MRI imaging, different medical diagnosis, etc. Fig 3 gives an idea of the literature survey process.

**RESULTS AND DISCUSSIONS**

Histopathology is the study of the biopsy samples by pathologist, using a microscope that is fixed on the glass slide [19]. Histopathological images are the digitized pictorial representation of the tissue samples captured from the stained slides with the help of microscope. The images under the microscope are basically captured under 4x, 10x, 40x and 100x magnifications. 4x magnification is generally referred to as the scanner view by pathologist which is used to check the distribution of cells in the biopsy sample. The 10x and 40x is referred to as low level and high level view where pathologists concentrate and observe the region of interest. 100x is obtained to study the cytological properties of the tissue samples. A tissue samples contains many inflammatory cells and regions together with the targeted region. Therefore extraction of region of interest by pathologist is done for digital image analysis by computer scientist. Texture analysis gives the spatial distribution of the cells in the tissue samples. For histological images it gives the association between the neighboring pixels relating to the smoothness, coarseness, roughness and/or roundness of the surface of the tissue sample. There are many predefined methodologies for texture feature extraction. Texture based classification [20-22] is used for childhood MB classification for classifying it into anaplastic and non-anaplastic subtype. Morphological analysis deals with the structure and shape of the nuclei feature to distinguish between normal and malignant cell. Most cytological classification uses morphology features to extract features in terms of eccentricity, perimeter, area, nucleus to cytoplasm ratio, length of major axis and minor axis etc. Many morphological studies have been carried out for areas such as oral, breast, cervix, renal etc. but no such work is available for childhood MB.
Image processing for histopathological samples has gained popularity in recent past. Digital Image analysis can give us more information relating to the biopsy samples than traditional pathologist review. Complex Information regarding survival, grading, longevity etc. can be answered by digital pathology. There are four basic steps (Fig 4) involved for automated histopathology. The first step is preprocessing of the samples which involves minimizing staining variations in generation of glass slides. Since slides are prepared by different individuals the difference in nature of staining arises. Also the light intensity for observation of images under the microscope varies from individual to individual. Therefore to leverage these differences preprocessing of the raw image data is necessary. Secondly feature extraction is performed to define the region of interest. The region of interest is the area from which the pathologist obtain information about the tissue samples. Features may include the physical perimeter of nucleus to cytoplasm ratio, Area, Diameter, Radius, Major and Minor axis and perimeter of nucleus, density of cells in the region, color chromaticity etc. However every feature might not be significant for a particular problem therefore third and important steps selection of important feature for evaluation. The fourth and finally step is classification of the sample using computer or statistical methods.
They performed their experiment on anaplastic and 300 non-anaplastic MB images. The classification accuracy was 91%. Features extracted were classified using a random forest classifier. The classification accuracy was 91%.

The study by Cruz-Roa et al. [20] was the most recent that we came across. They compared two CNN based models. The first one was Visual Geometry Group CNN with 16 layers. The second model was IBCa-CNN which was a 2 layer CNN. The features from these two models were used to classify between anaplastic and non-anaplastic MB using a softmax classifier.

Table 1: Summary of the studies on diagnosis of MB using computer methods

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Work</th>
<th>Segmentation</th>
<th>Feature</th>
<th>Classifier</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lai et al.</td>
<td>2011</td>
<td>Anaplastic and non-anaplastic MB</td>
<td>n/a</td>
<td>Haar Wavelet and MR8 filter</td>
<td>KNN</td>
<td>80%</td>
</tr>
<tr>
<td>Tchikindas et al</td>
<td>2011</td>
<td>Nodular MB</td>
<td>Hierarchical Normalized cut and random walker</td>
<td>Did not perform</td>
<td>Did not perform</td>
<td>83% (segmentation)</td>
</tr>
<tr>
<td>Lai et al.</td>
<td>2011</td>
<td>Anaplastic and non-anaplastic MB</td>
<td>n/a</td>
<td>Haar, Haralick and Laws textural features</td>
<td>Random forest</td>
<td>91%</td>
</tr>
<tr>
<td>Cruz-Roa et al.</td>
<td>2015</td>
<td>Anaplastic and non-anaplastic MB</td>
<td>n/a</td>
<td>IBCa-CNN</td>
<td>Softmax</td>
<td>89.8% and 76.6%</td>
</tr>
</tbody>
</table>

Lai et al. [22] performed a patch based classification with data collected from 6 patients out of which 3 patients had anaplastic and remaining 3 had non-anaplastic cells. They used 200x magnification for their work. The images were then divided into 100 patches of 500x500 each. In total they had 300 anaplastic and 300 non-anaplastic patches. Features were extracted using Haar, Haralick and Laws texture features. The features extracted were classified using a random forest classifier. The classification accuracy was 91%.

The study by Cruz-Roa et al. [20] was the most recent that we came across. They compared two CNN based models. The first one was Visual Geometry Group CNN with 16 layers. The second model was IBCa-CNN which was a 2 layer CNN. The features from these two models were used to classify between anaplastic and non-anaplastic MB using a softmax classifier. Results showed that IBCa CNN features showed much better performance than VGG-CNN with accuracy 89.8% and 76.6% respectively. They had 10 patient’s data with 5 anaplastic and 5 non-anaplastic.

All total we had four papers which had done work related to automated diagnosis of MB and all the work was based on binary classification. MB is a highly sensitive tumor and has a
multiclass classification with four subtypes. Therefore there is more scope of work relating to the malignancy.

Belsare and Mushrif [25] in their paper pointed out the challenge and analysis of histological images. Processing of histological images can be a lot more difficult than radiological images. Histological images have a lot of other cells than the targeted nuclei. Moreover, the segmentation methods used are based on individual datasets which may not work for other datasets. The reason is the use of different magnifications by different researchers for their study. One solution is to have a larger dataset, which is difficult as it depends on the availability of data. Also, the ground truth of nuclei detection is subjective of the individual pathologist. The architectural property of MB subtypes are very minute and requires experience and skill to evaluate such abnormalities. As diagnosis depend highly on its type it is very necessary to have accurate classification.

CONCLUSION

The evaluation by pathologist is mainly a subjective mental evaluation where the result always depends on the individual pathologist’s knowledge and experience. Childhood MB is a pediatric (embryonic) tumor and thus its severity is very difficult to diagnose from clinical aspects alone. Using computer aided diagnosis, childhood MB has been classified as anaplastic and non-anaplastic type only, till date. However according to World Health Organization it has four subtypes. Automated multiclass classification has still not been reported. The two extreme ends of MB according to the histological spectrum, namely nodularity and lager cell/anaplastic are associated with better and worst clinical outcome respectively. An increase in the grade of anaplasia is significantly associated with progressively worst clinical outcome. However neither increasing nor decreasing of nodularity or desmoplasia were related significantly with longer survival [23]. A proper subtype classification can have more accurate diagnosis for patients resulting in improved survivability. Though molecular classification with histopathology is used in developing countries for targeted therapy of the tumors, molecular study is still not possible in the region and country due to poor infrastructure and lack of technicians. Moreover molecular study also takes more time than histopathology. Automated histopathology will always have an unbiased outcome and is expected to be an add-on to the expertise of the pathologist. Accurate automated detection of class of the tumour is itself an achievement in this direction. Henceforth, Multiclass classification of childhood MB can help in accurate and proper diagnosis which will increase the longevity of patients. Further, automated analysis can also help us with the grading of the biopsy samples that has very minute difference between subtypes of tissue samples. Therefore developing a computer aided system for childhood MB histopathological subtype classification is more beneficial than only categorizing it into anaplastic and non-anaplastic variant. Ongoing research work for increasing the survival rate in neurology is currently prevailing and automated classification will be an added advantage.

Authors' contributions

Daisy Das had done the practical survey of the histological processing.

Dr. Lipi B. Mahanta supervised the whole idea of the work and was a conceptual support team member.

Dr. Basanta Kr. Baishya and Dr. Inamul Haque were from the neurosurgery department that gave us an insight of the data for MB tumors and also provided the clinical inputs.

Dr. Shabnam Ahmed gave the pathological information input.

Informed consent:

Informed consent was obtained from all individual participants included in the study.

Conflict of Interest

No conflict of interest.

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