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Conceptual Paper

Cytology for Veterinary Clinicians - An Introductory Note

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Introduction

Cytology (also known as cytopathology) was established as a diagnostic technique in 1960's and involves examining cells from bodily tissues or fluids to determine a diagnosis. Over the following decades it has branched out to other fields of medicine. Routine diagnostic tests of hematology, clinical biochemistry along with clinical examination many a times fail to give a definitive diagnosis. The cytological examinations not only help in disease diagnosis but also have it has prognostic value. Diagnostic cytology is classified into two major branches viz., exfoliative cytology and interventional cytology [1]. Exfoliative cytology is the study of cells naturally shed in body fluids and later examined under the microscope with or without centrifugation. Whereas Interventional cytology involves the intervention with the animal's body like fine needle aspiration to get a sample. At times, diagnostic cytology may not reveal a definitive diagnosis and thus has to be complemented with histopathology as a gold standard [7].

Cytological examination has the biggest advantage of speed and ease of sample collection combined with a rapid and clinically useful diagnosis of the ailments in the affected animals. It is an excellent cost effective and a minimally invasive diagnostic procedure that generally does not require surgery or any form of specialized or general anesthesia except for sedation or local anesthesia in some cases [3]. Despite all these advantages of diagnostic cytology, it is still under-utilized in India and stands a great scope for future expansion.

Collection and processing of samples for cytology

The basic steps involved in the cytological diagnosis include proper sampling, preservation and processing of the samples, staining and examination under the microscope for its interpretation. So proper sample collection forms the first critical step in cytology. The result of any attempt at cytological evaluation is greatly influenced by the quality of the specimen obtained and the way the samples are handled prior to evaluation. It's easy to interpret a slide that is prepared and stained well but even superb interpretative ability cannot compensate for failure in the critical steps of "sample preparation". Most specimens received in the laboratory are either as direct cell spreads (on slides) or as cell suspensions (fluids) [2].

Common techniques for collection of cytological specimens are, 1) Swabs, 2) Scraping, 3) Imprints and 4) Fine needle aspiration. Swabs - this technique is useful for the sampling of natural openings like oral, nasal and urogenital opening, fistulous tracts, ear canals and cutaneous open wounds. If the location for cytology is too dry, lightly moistening the cotton tipped swab with sterile normal saline improves cell retrieval. From the swab, the cells are transferred onto the glass slide by gentle rolling, rather than using side to side movement resulting in disruption of cells. In scrapping method, the blunt edge of the scalpel blade is used to scrape the affected site (usually the skin) till visible material is seen on the scalpel edge. The material so obtained with unidirectional smooth movements, blade is slowly applied onto the glass slide. Similar technique may be used for sensitive tissues such as conjunctiva, tongue and mesenchymal neoplasia, wherein the cell retrieval is generally poor. In case scabs/crusts are found over the lesions, they should be gently removed in order to improve cell retrieval and achieve better morphology. In situations where a large amount of blood or fluid or pus contamination is expected, the tissue should be blotted dry before attempting smear collection. Primary cancers are usually ulcerated

and may be associated superficially with inflammation and infection. Therefore, underlying neoplastic cells may remain masked are usually missed during imprint smears in such cases. In addition to neoplasia, the diagnostic cytology is useful for detecting the severity and type of inflammation as well as associated infectious etiologies [4].

Fine Needle Cytology/Fine Needle Biopsy is the best and most commonly used method for sampling proliferative lesions and masses. Generally, a thin needle of 21-24 guage attached with a syringe of 5-10ml. is sufficient for aspiration cytology of soft tissue masses. Sampling by fine needle cytology can be done either with aspiration or non-aspiration methods. a) Aspiration method: In aspiration procedure, the fine needle is inserted into the suspected mass without any air in the syringe, then the negative pressure is applied on the plunger to suck in the cells. While continuing to apply the negative pressure, the needle is redirected at 3-4 different angles to obtain representative cytology sample from different areas within the given mass. "Smaller and softer the mass, smaller the syringe used". The best aspirated material is when it is restricted to the hub of the needle and is not visible in the syringe. If too much blood is collected within the syringe during aspiration procedure, another attempt should be made away from the first aspirated site. Negative pressure is released prior to pulling off the needle from the mass. Immediately after the aspiration procedure is completed, the needle is removed from the syringe, air is drawn into the syringe and the needle is reattached firmly to the syringe. Lastly the aspirated material forms the hub of the needle is smeared onto the recipient slide, keeping the tip of the needle close to the slide. b) Non-aspiration method: In non-aspiration technique, the fine needle is directly inserted into the affected mass and redirected within the mass like a stab. It is good when the masses are highly vascular or having lot of blood or pus or fluid. Once the needle is removed from the mass, the positive pressure is applied through piston and the aspirated material is smeared onto the slide gently.

The dispatch of cytology samples to the laboratory should be rapid and in suitable containers having lids. Lids should be properly secured to prevent any leakage and specimens should be sealed in plastic bags. Glass slides should be kept in suitable slide boxes. All specimens and slides should be properly labeled with patient's name and number. After fixation of the smear, they should be transported in containers that protect against breakage of slides during transit.

The most preferred methods for smear making in diagnostic cytology is Squash preparation. In this method, the aspirate is placed on the recipient clean glass slide usually towards the center of it. A second spreader slide is placed at right angle to the recipient slide and then gently and smoothly moved over it by slow rotation so as to make two sister smears. Application of too much pressure or harsh movement during squash preparation is disruptive to the cells. Star fish preparation is usually made when the sample is liquid. The material is drawn outwards in a zig-zag manner in multiple directions with the help of a needle to make a stellate shape on the slide. This allows concentration of cells on the periphery of star fish smear. Blood smear technique is aspirated, if applied material is having consistency similar to blood. Angle in such cases is usually around 45 degrees between the recipient and spreader slides and the movement should be steady and smooth.

Staining

The examination of smears prepared requires proper staining to visualize the cells under the microscope. There are different stains available for routine cytological evaluation in veterinary practice. Most of these are Romanowsky-type stains such as Diff Quik stain, Geimsa stain, Wright's stain, Leishman stain, etc. Alternatively, staining kits like "Wright-Giemsa Stain Kit" and Papinocolaou are available commercially for use. Alternatively, some special staining like Toluidine blue, Periodic Acid-Schiff, Gram's stain and immunecytochemical stains are also used. Romanowsky stains are widely used for air dried smears throughout the world. Combination stains are considered better. However, Papinocolaou stain is considered the best, although it needs wet fixation in ethanol. Since it is time consuming and cumbersome, it is considered impractical in field conditions or for routine use. In many of the in-house labs., Diff-Quik stain is used for initial screening of the cytologic specimes, but it provides only preliminary details of cells.

Interpretation of cytological smears

Cytological interpretation requires a lot of experience to discriminate and give a proper and conclusive diagnosis. The foremost step in cytological interpretation is whether the lesion is inflammatory or neoplastic or reactive hyperplasia. Quite often, the neoplasia is complicated with secondary inflammation. In inflammatory lesions, the cells are differentiated according to their morphology into various lineages. The predominant cell population

is taken into consideration while classifying inflammation. The most difficult part of cytology is distinguishing between the reactive and neoplastic cells particularly those of mesenchymal and epithelial origins. Cytological diagnosis of neoplastic conditions is made on the basis of typical cyto-morphological features [5]. However, information regarding the location/size of the lesion and the method of specimen collection are important in determining the diagnosis.

Another important point to be kept in mind in the diagnosis of a tumor with inflammation is to consider a granulomatous lesion as the other differential. Macrophages in tissues can be large and appear "epithelial," in fact these types of macrophages are referred to as "epithelioid macrophages." If some of these cells in question have vacuoles of various sizes, and have phagocytosed something, they are probably macrophages and not tumor cells. When there are numerous mononuclear cells with minimal or no inflammation, then it may be a tumor, hyperplasia, or a normal structure. Normal structures generally yield few cells whereas usually numerous cells exfoliate from neoplastic lesions. The more uniform the mononuclear cells, the more likely it is a normal structure, or at least "benign." This is usually not a problem as clinician does not go around aspirating normal structures. The cytological features of the cells are evaluated to classify the tumor as benign or malignant and as to its tissue type, i.e., epithelial, mesenchymal, or round cell [6]. An attempt can be made to assess the potential malignancy of the tumor by identifying cytological criteria of malignancy, the most important of which is pleomorphic nature of the cells. If the cells are numerous, but resemble a normal tissue/organ, their appearance must be critically assessed to decide between normal (perhaps the lesion was missed), benign proliferation (focal hyperplasia) or a benign tumor. This is really only a dilemma when aspirating internal organs. For dermal lesions forget hyperplasia and normal structure, you are aspirating a lump and it is not normal. Decide this: is the dermal lesion inflammatory or not inflammatory. If it's not inflammatory then decide if it is a round cell, mesenchymal or epithelial cell tumor, and then try to decide if it is benign or malignant.

Based upon cytologic interpretation, the tumours are classified into 4 basic subtypes i.e., round cell tumours, epithelial tumours, mesenchymal tumours and melanocytic tumours. Round cell tumours are best diagnosed at diagnostic cytology and correlate excellently with histopathology. The cell retrieval in such tumours is excellent and differentiation is rather easy. Based upon the morphology, these are further classified into Lymphomas, plasma cell tumours, mast cell tumours, transmissible venereal granulomas and histiocytic tumours. These generally consist of moderately large round cells with variable cytoplasmic content vacolization and granularity. Epithelial tumours have generally good cell retrieval and cells are seen in cohesive clusters. The cytology of glandular tumours is similar to that of epithelial tumours except that the latter have secretory content or vacuoles in their cytoplasm. Tumours of mesnchymal origin have generally poor cell retrieval and at times difficult to diagnose. Whereas melanocytic tumours particulary amelanotic melanomas are most difficult to diagnose cytologically because they may show all the three patterns resembling round cells, epithelial cells and mesenchymal like cells.

Limitations in cytology

A major constraint in the cytological diagnosis is the lack of experience even though it's a simple and effective technique, lack of experience results wrong diagnosis many a times. Some of the other limitations of diagnostic cytology are that definitive morphologic diagnoses may not be possible, tumors cannot be graded, margins cannot be evaluated, and quality of preparations can greatly influence the final interpretation. One of the key limitations in diagnostic cytology is that a fine needle is poked into a big mass in a blind folded manner, which may not yield the representative sample size and cell population [7].

Conclusion

Cytopathology, whether exfoliative or invasive, is a cost effective, fast, simple and labour easy technique which can be effectively used in the diagnosis of pathological ailments of varying nature in animals. Though cytology is being regularly used in humans especially for the purpose of suspected neoplastic conditions, its use in field of veterinary science is restricted but started gaining importance in the present times. Proper sampling and right interpretation of slides are acting as the major constraints mainly due to lack of experience in these aspects. But with the advent of time and advancement in the technical aspects, diagnostic cytology is bound to play an important role in the diagnosis of diseases of animals. Further the importance of cytopathology and its diagnostic accuracy needs to be reviewed and analyzed regularly in detail to file the research gap for better future outlook.

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