Can direct immunofluorescence testing still be accurate if performed on biopsy specimens after brief inadvertent immersion in formalin?

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Background: Direct immunofluorescence is useful in the diagnosis of autoimmune, vesiculobullous, and connective tissue diseases. Michel medium is typically indicated for transport, but clinicians may inadvertently place samples into formalin.

Objective: We set out to determine the amount of time that specimens can remain in 10% buffered formalin and still retain their diagnostic properties.

Methods: Biopsy samples were examined from cases with established diagnoses of bullous pemphigoid (n = 12), dermatitis herpetiformis (n = 6), and pemphigus vulgaris (n = 6) and exposed to formalin for time points ranging from 2 minutes to 4 hours.

Results: We found that immunoreactants were detectable in the majority of samples when subjected to 2 minutes of formalin exposure. Dermatitis herpetiformis and pemphigoid samples retained immunogenicity for 10 minutes, whereas pemphigus showed reduced immunogenicity for all samples studied. A nonimmunologic nuclear fluorochroming pattern was noted in some of the specimens after formalin immersion.

Limitations: Sample size, only examining 3 disease processes, and samples already having been in Michel medium were the major limitations in the study.

Conclusion: In direct immunofluorescence studies, formalin exposure to biopsy specimens causes two types of artifactual changes: (1) the shortest exposure (2 minutes) causes complete loss of diagnostic markers of pemphigus; and (2) prolonged exposure changes tissue to a form that allows fluorescein-labeled antibodies to give fluorochroming reactions of nuclei (which can be mistaken for in vivo antinuclear antibody reactions of lupus erythematosus). After time intervals of 10 minutes to 2 hours, direct immunofluorescence studies of proven cases of bullous pemphigoid and dermatitis herpetiformis retained variable levels of specific reactivity. (J Am Acad Dermatol 2011;65:106-11.)

Key words: antinuclear antibody; bullous pemphigoid; dermatitis herpetiformis; direct immunofluorescence; formalin; Michel medium; pemphigus.

Direct immunofluorescence (DIF) is helpful in the diagnosis of vesiculobullous and connective tissue diseases.[1,2] Proper handling of tissue specimens is important and Michel medium is considered to be an optimal transport medium.[2,3] Other techniques have been tried to maximize both quality of immunofluorescence examination and ease of use, but Michel medium remains widely used.[4,5] Formalin is used for routine transport of biopsy specimens for conventional histologic examination. Although the use of formalin has a negative effect on levels of

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immunofluorescence in both skin and oral mucosal biopsy specimens in comparison with fresh-frozen tissue, some relevant immunofluorescence remains.[6,7] Clinicians and their staff occasionally place tissue samples into formalin and later decide that DIF evaluation may be needed. We sought to define how long tissue could remain in formalin before levels of immunofluorescence decline, impairing accurate diagnosis.

CAPSULE SUMMARY

- We examined the effect of formalin exposure on direct immunofluorescence biopsy specimens.
- Short exposure to formalin (as little as 2 minutes) might be detrimental to the interpretation of some biopsy specimens.
- After longer exposure to formalin some samples could be read as false-positives for lupus erythematosus.
- Dermatologists and dermatopathologists must be aware of the adverse effects of formalin on direct immunofluorescence samples.

METHODS

Specimens

A total of 24 biopsy specimens previously received at our laboratories and with positive DIF findings for immune deposits were evaluated. These included 12 biopsy specimens positive for basement membrane zone deposits (bullous pemphigoid or other autoimmune diseases with such deposits), 6 positive for granular junctional/papillary dermis deposits of IgA (dermatitis herpetiformis), and 6 positive for epidermal intercellular deposits (pemphigus).

Controls

Three to 4 sections were processed using the routine procedure[8,9] for DIF testing before the specimens were placed into formalin. The intensity of fluorescence of the disease-specific immune deposits was graded as follows: negative, +/− (doubtful), + (weak), ++ (moderate), +++ (bright).

Experimental specimens

Each biopsy specimen was designated for a specific time point to be placed into 10% formalin. These were 2, 10, 30, 60, 120, and 240 minutes. The smallest time point of 2 minutes was chosen to take into account the time it might take to realize the error, remove the specimen, procure the Michel medium specimen container, and put the biopsy into the container. Whether the formalin will continue to have an effect after the specimen is removed until it is placed into the new container is unclear; nevertheless, we took this into account as the formalin could still react with the biopsy specimens. Two biopsy specimens with positive basement membrane zone deposits were designated for each time point and one specimen for each time point for biopsy specimens positive for dermatitis herpetiformis and pemphigus. The specimens were stored in Michel medium for 24 hours after removing them from 10% formalin. Michel medium is composed of 25 mL 1 mol/L potassium citrate buffer (pH 7.0), 50 mL 0.1 mol/L magnesium sulfate solution, 50 mL 0.1 mol/L N-ethyl maleimide solution, 875 mL distilled water, and 550 g ammonium sulfate with a final pH between 7.2 and 7.4 (stored at room temperature). These were then processed for DIF using the same procedure as above. Preparations were examined by two investigators for disease-specific immune deposits and the intensity of fluorescence was graded as described above. In addition, background and other nondisease-specific staining was also noted.

RESULTS

Biopsy specimens

As described above, biopsy specimens were divided into 12 cases positive for IgG and/or C3 linear basement membrane zone deposits (bullous pemphigoid or other autoimmune diseases with such deposits), 6 cases positive for granular junctional/papillary dermis deposits of IgA (dermatitis herpetiformis), and 6 cases positive for epidermal intercellular deposits (pemphigus). Samples varied in immunofluorescence levels and were rated 1+ to 3+ (Tables I to III). Biopsy specimens, particularly those positive for epidermal intercellular deposits, lost immunogenicity after storage. We found that biopsy specimens that were stored for longer than 2 years lacked immunogenicity in all 3 disease processes (data not shown).

Pemphigus

Epidermal intercellular deposits were demonstrated in all control specimens that were used (Table I). One specimen was examined for IgG4 subclass staining (which was subsequently immersed in formalin for 1 hour). All time points (with the exception of that examined for IgG4 subclass) resulted in a nondisease-specific cytoplasmic staining pattern that prevented determination of intercellular deposits. In addition, all samples exposed to formalin for 10 minutes or longer (with the exception of that examined for IgG4 subclass) displayed nondisease-specific nuclear staining or fluorochroming that also precluded examination