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Research Article

A Study of Efficacy of Rose Bengal and Lissamine Green Stain in Dry Eyes

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ABSTRACT
Background: Ocular vital dyes are part of determining the disease of dry eye (DED). Rose bengal (RB) and lissamine green (LG) vary in the tolerability of patients and cell-interaction, but comparative, practice-based evidence presented in regular clinics is useful. It was a cross-sectional study of staining efficacy and tolerability of RB and LG in a hospital setting and based on the Van Bijsterveld scale.
Methods: Individuals with DED who are adults (>=18 years old) and visit two tertiary centers were recruited (n=64). The exclusion criteria were dye hypersensitivity or lack of consent. One group (A) consisted of consecutive users, and the other one (B) involved sequential users. The findings were demographics, I (no anesthesia) with slit-lamp. The main outcomes included (i) patient-reported tolerability (with no complaint vs irritation/burning/itching/stinging), (ii) observational sensitivity by Van Bijsterveld intensity (mild/moderate/severe) and (iii) anterior chamber penetration. Such statistics encompassed t-tests and Chi-square.
Results: There was no difference in the mean age between the groups (LG 44.31±13.56 vs RB 45.29±12.80 years; p=0.76). The distribution of sex was comparable (p=0.45). Tolerability was significantly improved between LG and RB (no complaints 84.37 vs 37.50). RB has reported a moderate-to-severe intensity staining (13/9/10 counts=mild/moderate/severe) in comparison with LG (25/7/0); chi-square=14.04, p=0.0008). There was no anterior chamber penetration in any case of either group (0%). Another finding was that blood pressure and random blood glucose were similar among groups (p>0.05).
Conclusion: Under normal conditions of DED practice, LG offers significantly more patient-friendly without reducing the detection in mild-moderate disease, but RB more effectively shows higher-intensity staining but at the expense of a very high level of discomfort. The results correlate with the experimental evidence that RB is more cytotoxic/irritating, whereas LG is more tolerable and is advised in any existing diagnostic algorithm. Through pragmatic means LG is better to be used in the first line of ocular surface staining leaving RB to certain situations whereby there is the need to detect severe epithelium in the form of keratinized/devitalized epithelium.

Keywords: Dry Eye Disease, Lissamine Green, Rose Bengal, Ocular Surface Staining, Van Bijsterveld Score, Tolerability.

INTRODUCTION

Dry eye disease (DED) is a multifactorial ocular surface and tear film disorder with a set of symptoms and evidence indicating tear film instability, elevated levels of hyperosmolarity, inflammatory and neurosensory abnormalities [1]. It is advised that all cases to which the diagnosis could be applied should undergo international consensus-based diagnostic battery (symptom screening, tear stability testing, and ocular surface staining, when possible with fluorescein and cornea and lissamine green (LG) and conjunctiva/eyelid margin, respectively) [2]. The vital dyes are still in the spotlight since they are cheap, fast and informative within a busy clinic [3].

Traditionally, rose bengal (RB) and LG were employed to compare the devitalized cells and areas deprived of mucus. Van Bijsterveld suggested an 0-3 rating of nasal conjunctiva, cornea, and temporal conjunctiva (maximum 9) that is still common [4,5,6]. Norn used LG to stain degenerate cells and mucus and spared healthy epithelium, effectively introducing ophthalmology to LG [7]. In comparison to that, the mechanistic work by Feenstra and Tseng also helped to understand that RB is not necessarily vital: it may stain living cells that are not protected by tear film components (e.g., mucins, albumin) and can undergo dose-dependent, mainly nuclear uptake with possible effects because of photodynamic processes [8].

Clinical observations Walsh and Wellness (2006: 379) report that clinical observations of LG and RB are alike in terms of offering similar diagnostic data regarding ocular surface disease; however, LG is generally better tolerated compared to RB [9,10]. In modern reviews of grading scales, a move away in favor of routine use of RB due to discomfort and possibly toxicity is also reported, in favour of LG to stain conjunctiva in DED [6]. Refinements in the methods have optimized the parameters throughout the staining concentrations of LG in the modeling stage and instillation at the highest contrasted change [11].

Regardless of these improvements, real-life comparative information of regular hospital clinics where patient comfort may affect the feasibility of tests can be useful. We prospectively compared the effectiveness (observational sensitivity with Van Bijsterveld intensity) and the tolerability of LG and RB in adults with DED in two tertiary centers. Our hypothesis was that, at the same patient comfort, LG would show the same or better patient comfort with reduced detection in mild-to-moderate disease, but RB could focus more on severe staining intensity, like those reported by the molecular mechanochemistry of LG with damaged epithelium and mucin loss [8,11].

MATERIALS AND METHODS

Study design and setting

Our study was carried out in a cross-sectional and hospital-based research in the Regional Institute of Ophthalmology (Sarojini Devi Eye Hospital) and in Gandhi Hospital. The research time frame was located in regular outpatient clinics; all practices were carried out in accordance with the Declaration of Helsinki. Institutional ethics approval was obtained prior to initiation and informed consent was written out and signed by all participants.

Participants

Adults above 18 years old with clinical diagnosis of DED established through the symptoms and slit-lamp inspection were recruited serpentinely (n=64).

Hypersensitivity to RB or LG and the inability to give voluntary consent (excluding intellectual disability, altered consciousness, pediatric age group) were exclusion criteria.

Allocation and interventions

The respondents were divided into two equal groups (A and B; 32 each). Group A was stained using LG on the ocular surface and Group B with RB. Dye strips with 1.5 mg LG (Group A) or RB (Group B) that were commercially available had applied the squares to the inferior fornix once moistened with sterile saline. Slit-lamp biomicroscopy examinations were done using trained ophthalmologists wearing masks during grading that were graded as group assignment.

Clinical assessment

The medical history, medications, and demographics were taken down. Snellen (visual) acuity, general examination, and anterior segment examination were done. Grade of Aqueous deficiency was graded using Schirmer test (without anesthesia). Systemic status was described by measuring blood pressure (BP) and random blood sugar (RBS).

Outcomes

1. Please, report the immediate tolerability after instilling the dye (no complaint vs irritation/burning/itching/stinging);
2. Observational sensitivity Van Bijsterveld intensity scores (mild, moderate, severe) through rating procedures upon the cornea and bulbar conjunctivae;
3. Safety outcome of anterior chamber penetration (yes/no).

Ethics, safety, and data integrity

Unfavourable sensations were recorded. Topical staining was not done to help to avoid any topical anesthesia as it is required to do routine test sequencing [2]. There were no investigational products.

Statistics

Mean+SD were used to summarize continuous variables and compared with independent-samples t -tests. Chi-square tests were used to compare categorical variables. A p-value of below 0.05 was taken to be statistically significant. Complete Cases Only complete cases were analyzed (n=64).

RESULTS

Participants and baseline characteristics

The mean ages of 64 subjects did not differ in two groups (LG 44.31±13.56 years; RB 45.29±12.80; p=0.76). The total number of females was 57.81%. The greatest age-groups were 31-50 years (=53%) and 30-34 years (=34%). The I values of Schirmer had mild-severe aqueous lack of invasion across cases. There was no difference between groups in BP and RBS (all p>0.05).

Tolerability

The percentages of LG being better than RB are significant: no discomfort was noticed in 84.37% and Irritation/burning / itch was in 15.63%.

Observational sensitivity and intensity distribution

RB exhibited a right shift to higher staining intensity (mild/ moderate/ severe 13/9/10) than LG (25/ 7/ 0). The chi-square test (anticipated 19/19 per cell, charges 8/8 and 5/5 for mild, moderate, and severe respectively) showed that there are differences in the distributions of intensities (2=14.04, p=0.0008). In-sex stratification was no less common.

Safety

No eye in either group showed anterior chamber penetration of dye (0%).

Table 1. Demographic Profile

Variable	Lissamine Green (n=32)	Rose Bengal (n=32)	p-value
Age, mean ± SD (years)	44.31 ± 13.56	45.29 ± 12.80	0.76
Female, n (%)	17 (53.12)	20 (62.50)	0.45
Male, n (%)	15 (46.88)	12 (37.50)	—

The age and sex distribution between the two groups were well balanced so that the demographics did not confound the study. The absence of between-group differences (p.05 in the case of the variables age and sex) is favorable to the internal validity of the following comparisons. This is in line with the known DED epidemiology as the female percent is majority at about 58. The very similar mean age indicates the similarity of the chronicity and risk of the diseases (e.g., perimenopausal condition, systemic comorbidities), which allows a reasonable evaluation of comparative staining results and patient intolerability.

Table 2. Severity Distribution (Overall and By Dye)

Grade of DED	LG (n)	RB (n)	Total
Mild	5	15	20 (31.25%)
Moderate	17	12	29 (45.31%)
Severe	10	5	15 (23.44%)*

The majority of the participants were having moderate disease with a significant number of severe cases. Distribution provided LG arm cases were more severe and RB arm cases were milder, which might bias crude cases distributions. Nonetheless, further analysis through a chi-square to assess observed staining intensity (not classified severity in advance) indicated there was significant dye-related variation which indicated that the intrinsic staining behaviour in both RB and LG played a significant role in the observed patterns beyond baseline grade.

Table 3. Patient-Reported Tolerability

Dye	No complaints n (%)	Irritation/burning/itching n (%)
Rose bengal	12 (37.50)	20 (62.50)
Lissamine green	27 (84.37)	5 (15.63)

LG was also significantly better tolerated, and the percentage of tolerance was more than four times greater and the presence rates of adverse feelings were 4 times lower when it was in relation to RB. These variations are in line with mechanistic and clinical literature that revealed RB to be intrinsically irritating and also phototoxic, with LG being relatively non-cytotoxic and highly comfortable [810,11]. Pragmatically, the comfort profile of LG enhances practicability of the standard clinics and longitudinal trials in which frequent staining is required

Table 4. Observed staining intensity by dye (Van Bijsterveld categories) with chi-square test

Intensity	Rose Bengal (n)	Lissamine Green (n)	Expected (RB, LG)	χ^2, p-value
Mild	13	25	19, 19	χ^2=14.04, p=0.0008
Moderate	9	7	8, 8	
Severe	10	0	5, 5	

RB had a greater fugitive distribution to greater intensity staining with more moderate and severe, but LG had concentrated in the mild with no severe flavors. The strong chi-square

value refutes a false difference of the staining distributions. It lasts longer in vivo and can more easily characterize devitalized/keratinized epithelium and mucin deficiency areas -where there is clear evidence of a severely fragile mucous surface whereas LG would provide much superior visualization of milder/conjunctival disease and be more comfortable to use.

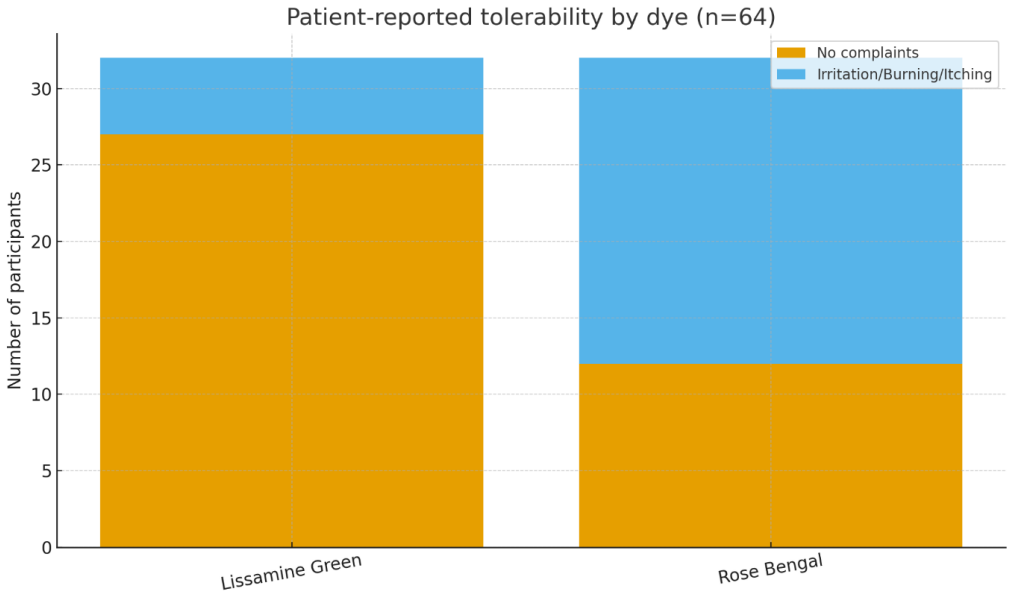

Figure 1. Patient-Reported Tolerability by Dye (Stacked Bars; N=64)

The stacked bar display shows the high level of comfort LG is much less irritating: in the vast majority of cases, the eyes covered by LG suffered no consequences, but RB provoked irritation very often. This is in line with the controlled trials illustrating the superior tolerance of LG on patients compared to RB and mechanistic data on cytotoxic/photodynamic exerted by RB on unprotected epithelium [8-10]. Practically, tolerability may be the key to test compliance and the repetition of a test; hence, the LG profile reveals the use of the technique as the consistent first line dye to be used in staining the ocular surface in DED.

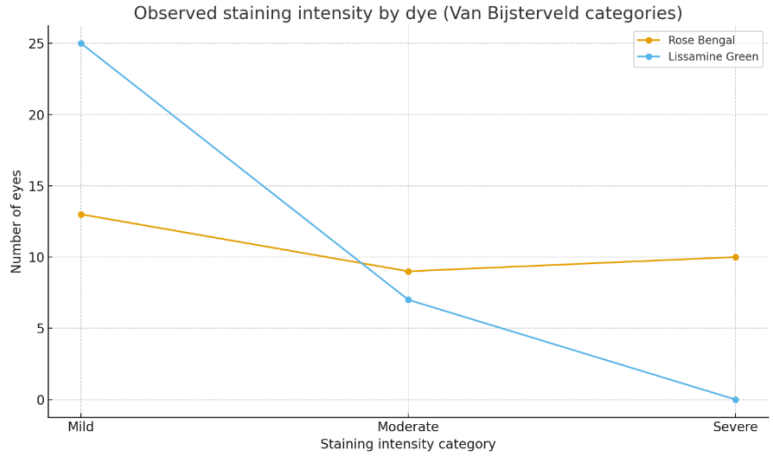

Figure 2. Observed Staining Intensity by Dye (Line Plot across Van Bijsterveld Categories)

RB exhibits flatter curve whereby those with moderate and severe intensity have more counts as compared to LG which has its maximum with mild intensity and zero severe. These trends indicate that RB is prone to staining the devitalized or keratinized epithelium and mucin-free zones, which is consistent with its staining mechanism, and LG will give sensitive results in previous or milder disease with a better patient experience [8 10 and 11]. Due to this, selection of dyes can be

specifically geared towards clinical suspicion and diagnostic objectives.

Safety outcomes.

No case demonstrated anterior chamber penetration for either dye (0%), and systemic parameters (BP, RBS) were comparable (all $p>0.05$), supporting the procedural safety of both dyes in this setting.

DISCUSSION

Cross-sectional analysis, in this real-life situation, LG was significantly more tolerable than RB, whereas RB generated staining distributions that were more intense. These results are supported by mechanistic and clinical literature. It was demonstrated by Feenstra and Tseng that RB stains cells that have lost protection via the loss of the preocular tear film (e.g., mucins/albumin) and can have photodynamic effects together with dose-dependent, predominantly nuclear uptake, which is likely the basis of the elevated discomfort and inclination to accentuate severely compromised epithelium [8]. Conversely, devitalized or membrane-damaged conjunctival and corneal epithelial cells and mucus, but not the healthy proliferating epithelial cells are preferentially stained with LG and have been linked with low cytotoxicity [7,910,11].

The similarity between our tolerability findings and that of the classic Ophthalmology trial by Manning et al, is that LG is much more comfortable than RB and they provide equal clinical information in the management of the disease, the keratoconjunctivitis sicca [9]. Ocular surface staining techniques are gradually being substituted by LG over RB in routine clinical practice due to its increased comfort and safety [6], and TFOS DEWS II diagnostic guidelines give LG as the conjunctival/eyelid margin stain of choice when used as a supplement to preliminary screening tests [2]. Standardized LG parameters (e.g., 1% solution, timing) further (maximization) studies are supported by optimization studies when maximizing diagnostic yield [11].

In contrast to the intensity observed, our chi-square analysis revealed that there were more moderate-severe conditions in RB compared to mild intensity in LG that included zero conditions of severe cases. Even though the difference was observed despite a certain imbalance of the pre-classified table regarding the grade of dry eye, it is possible to assume that indeed the dyes accentuate something different. The trend is a reflection of previous findings that RB attends to subregions of mucus deficiency/keratinization and can emphasize a further progressive mucosal damage [8,10,12], whereas LG is more focused on defining the devitalization of the conjunctival surface and the onset of the disease [7,9,11]. RB can continue to add to the information in particular situations, e.g., severe cases of keratoconjunctivitis sicca, keratinization of the mucus, or when assessing the presence of mucus abnormalities, all of which are reflected in its use in history [12,13]. Nonetheless, a regular dependence on RB is limited by the pain/symptom and possible cytotoxicity/phototoxicity risks [6,8,10,12].

Our safety data are hopeful: neither dye was excreted in the anterior chamber and there were no systemic issues which implies the normal procedure. However, the indication is that a significantly greater symptom burden with RB is worth highlighting within women with ocular surface hyperalgesia when making repeated serial measurements.

Clinical implications: In general DED clinics, the default LG ocular surface dye is: it is not painful, sensitive to mild-moderate disease and similar with present diagnostic algorithms [2,6,9,11]. RB could be reserved in the event of specific indications in when ambitious epithelial harm or mucus insufficiency is a possibility and when the clinician thinks that the improvement in diagnostic value will be higher than the comfort disadvantages.

Limitations: It was a cross-sectional and non-randomized study where allocation was done based on group, as opposed to random allocation; a slight imbalance in baseline grades category could confound intensity distributions, although even without randomizing observed intensities, statistical analysis of observed intensities found significant dye-related differences. Masked examinees scored Van Bijsterveld qualitatively and did not have Inter-grader-rater reliability. We omitted symptom questionnaires (e.g., OSDI/DEQ-5), and tear film measurements (e.g., non-invasive breakup time, osmolarity) which were suggested by TFOS DEWS II [2]. The sample size is not large enough to perform subgroup analyses but is sufficient in case of large effect sizes.

Research implications: Randomized, masked, split-eye, future studies would be required to integrate standardized LG protocols [11] with TFOS conforming diagnostic battery [2] or

pheonometric targets of image-analysis, studies could help answer whether LG is sufficient in most cases, and identify in what situations RB also adds information. The relative cost-efficiency and self-report measures of longitudinal courses of treatment would also provide evidence-based tests of treatment.

CONCLUSION

This study led to LG provided significantly better patient comfort at tertiary clinic patients with DED in comparison with RB and with moderate severity staining, whereas the latter was identified at the expense of a much larger amount of discomfort. There was no anterior chamber penetration in any of the two dyes. These results, which are consistent with mechanistic and guideline sources, suggest lissamine green as the initial ocular surface dye in standard DED testing, with rose bengal only used in select instances with selective accentuation of devitalized/keratinized epithelium or mucin-acidic foci which have a diagnostic point. Wider randomized, split-eye studies that will introduce TFOS aligned measures and image-analysis endpoint warrant to specialize dye selection regimes throughout the range of DED severity.

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