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Research Article
A Comparison of Immunomodulatory Monotherapy and Combination Therapy for Recurrent Aphthous Stomatitis

Yuan Xue, Jiyan Liu and Wenfeng Zhao

Department of Stomatology, PLA Army General Hospital, 100700 Beijing, China

Abstract

Background and Objective: Systemic steroids are used to prevent and heal mouth ulcers. However, ulcers often reappear after steroid withdrawal. The toxicity of systemic corticosteroids impedes their chronic use; corticosteroid-sparing agents must be employed instead. Here, the study evaluated the efficacy and safety of levamisole or prednisolone alone and a levamisole/prednisolone combination, in patients with recurrent aphthous stomatitis (RAS). **Materials and Methods:** Patients with RAS received levamisole 150 mg/day (Group L, n = 100), prednisolone 15 mg/day (Group P, n = 100) or levamisole 150 mg/day plus prednisolone 15 mg/day (Group L+P, n = 100), for 6 months. The study recorded ulcer number and size, pain intensity and serious and non-serious adverse events. The paired samples t-test and chi-squared test were used to define significant between group differences (with 95% confidence intervals). **Results:** At the end of 6 months, all patients exhibited reductions in both ulcer sizes and pain (both $p \leq 0.05$). Neither ulcer number nor size differed between patients given combination therapy and those receiving monotherapies ($p \geq 0.05$ for both). Non-serious adverse events for up to 6 months were more common in patients on the combination therapy than in those receiving the monotherapies (all $p \leq 0.05$). **Conclusion:** Therefore, it was concluded that combination therapy and the monotherapies exhibited similar efficacy. However, combination therapy was associated with more adverse events.

Key words: Immunomodulators, mouth ulcer, numeric rating scale, recurrent aphthous stomatitis, maxillofacial biology

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Corresponding Author: Wenfeng Zhao, Department of Stomatology, PLA Army General Hospital, 100700 Beijing, China Tel/Fax: 0086-13810850108

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Recurrent aphthous stomatitis (RAS) and recurrent aphthous ulcers (RAUs) are common mucosal conditions associated with painful ulcerations in the oral cavity (Fig. 1)[1]. Approximately 20% of the general population suffers from RAS but the prevalence ranges from 5-50% based on nationality, race and socioeconomic status[2–4]. The condition is more prevalent in females (up to 25% of the population)[5–7]. RAS may be caused by trauma, microbial infections, folic acid and B-complex deficiencies, immunological factors, psychosocial stress and allergy to food constituents[1]. To date, most research has focused on immunological factors. However, the cause of RAS remains to be determined[8].

RAS lesion formation is associated with a cell-mediated immune response, the generation of T cells and production of tumor necrosis factor-alpha (TNF-α), which plays a significant role in disease development[2,9,10]. The TNF-α mediates endothelial cell adhesion and neutrophil chemotaxis, triggering inflammatory processes that cause ulceration[9,10]. No definitive cure for RAS is known. However, analgesics, antimicrobials, corticosteroids and immunomodulators (alone or in combination) have been used to prevent and cure RAS. Of the immunomodulators, levamisole is an immunopotentiating agent that is particularly useful in treating RAS[11].

The side-effects of levamisole are minimal when the recommended doses are employed; the drug does not affect the cellular or humoral immunity of RAS patients. Levamisole enhances the activity of T-suppressor cells *in vitro*, inducing immunosuppression that effectively reduces RAS[12,13]. Levamisole restores the normal $CD4^+/CD8^+$ cell ratio and increases immunoglobulin A (IgA) and IgM production, eliminating aberrations in cellular and humoral immunity, alleviating pain, shortening ulcer duration and increasing the disease-free interval[2,13].

Systemic steroids have also been useful to treat RAS; few side-effects are apparent when the doses are appropriate[14]. RAS patients on prednisolone exhibit minimal symptoms. However, ulcers often reappear upon steroid withdrawal. The toxicity of systemic corticosteroids limits their chronic use; thus, corticosteroid-sparing agents must be employed[15,16]. Prednisolone has been suggested to suppress granulocyte migration to the site of tissue injury, phagocytosis, prostaglandin/leukotriene synthesis and cellular immunity[17]. To date, only levamisole[12,13] and prednisolone[16] monotherapies have been studied. The addition of levamisole to prednisolone afforded better control of RAS manifestations and the combination was relatively safe. Levamisole ensures longer-term remission and a rapid clinical response.

Fig. 1(a-b): Recurrent aphthous stomatitis on the interior oral mucosa (a) Regular view and (b) Microscopic view

Prednisolone and levamisole are both immunosuppressive agents but act via different pathways on distinct lymphocyte subgroups[18].

The objective of the study was to evaluate the efficacy of levamisole and prednisolone monotherapies and the combination therapy, in RAS patients. A secondary endpoint was evaluation of serious and non-serious adverse events developing over 6 months of continuous intervention.

MATERIALS AND METHODS

Materials: Levamisole (Dicaris, 50 mg) tablets were purchased from Johnson and Johnson Ltd., USA. Prednisolone (Wysolone, 5 mg) tablets were produced by Wyeth Ltd., China. Placebo tablets were purchased from Wuhan Uni-Pharma Bio-Tech Co., Ltd., China.

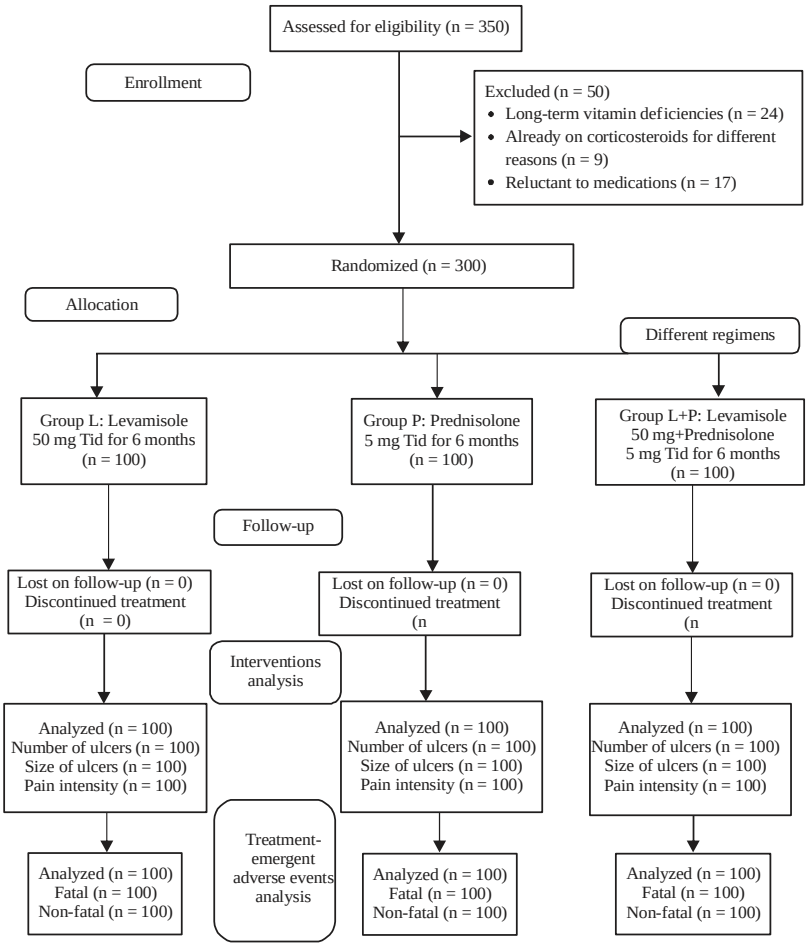

Fig. 2: Planning chart for evaluation of therapies in recurrent aphthous stomatitis (RAS) patients. Total number of patients evaluated in terms of eligibility: 300; hypothesized percentage efficacy frequencies: 90 ± 5%; confidence level: 95%. Tid: three times daily

Table 1: Demographic characteristics of all enrolled patients

Patient characteristic	Group L (n = 100)	Group P (n = 100)	Group L+P (n = 100)
Age (Mean±SD) (years)	27.93±2.42	28.86±3.03	29.18±2.81
Gender			
Male	53 (53)	50 (50)	54 (54)
Female	47 (47)	50 (50)	46 (46)
BMI (kg m^{-2}) (Mean±SD)	25.4±3.0	25.7±2.3	26.1±4.5

Data are shown as numbers (%), BMI: Body mass index, $p \geq 0.01$ for age, the male: Female ratio and BMI between Groups L vs. P, Groups P vs. L+P and Groups L vs. L+P

Study design and setting: This study was conducted in the PLA General Hospital, Beijing, China, between May, 2015 and April, 2017. All patients gave written informed consent. The Ethics Committee of the hospital approved the experimental protocol and the study followed all relevant ethical guidelines mandated by Chinese law[19].

Inclusion criteria: A total of 350 patients aged >18 years, of either sex, with a medical history of RAS at least 6 months in duration, maxillofacial pain, mouth discomfort and who were willing to participate, were enrolled. All patients had developed resistance to other steroid therapies (topical or systematic) at least 2 months in duration. Patients were randomized using a computer-based algorithm (Fig. 2)[20] to receive levamisole monotherapy (Group L), prednisolone monotherapy (Group P) or levamisole plus prednisolone combination therapy (Group L+P). All tablets were identical in appearance (Table 1).

Exclusion criteria: Patients with long-term vitamin deficiencies, who were already taking corticosteroids or who were reluctant to take medication, were excluded. Those with RAS attributable to chronic systematic diseases, who had already received at least one study drug, who had exhibited allergy to either drug during the past 3 weeks or were unresponsive because of a psychiatric illness or severe pain, were excluded. Those with altered blood cell counts or B-complex levels, gastrointestinal disease, diabetes, thyroid disease, Crohn's disease, celiac disease or dental caries or who were parafunctional, were also excluded.

Interventions

Group L: One placebo and one levamisole 50 mg tablet three times daily after food for 6 months.

Group P: One placebo and one prednisolone 5 mg tablet three times daily after food for 6 months.

Group L+P: One levamisole 50 mg and one prednisolone 5 mg tablet three times daily after food for 6 months.

The treatment periods commenced at the baseline (BL) visits. All patients visited the hospital monthly until 6 months had expired[15].

Outcome measures: The following parameters were measured in all patients, at BL and at the end of every month up to 6 months, by a single investigator blinded to patient treatment:

- **Ulcer numbers:** Ulcers were counted and numbered and the locations were identified on diagrams of the oral cavity[14]
- **Ulcer sizes:** Ulcer sizes were measured using calipers (Plastic Digital Caliper Hot, 0-150 mm, Yuyao Zhenggong Precision Measuring Tools Co., Ltd., China). The largest ulcer was considered representative of each assessment[14]
- **Pain intensity:** Pain intensity was assessed using a 101-point Likert numerical rating scale (NRS) ranging from 0-100: 0 reflected no pain and 100 the worst possible pain[14]
- **Safety:** Serious and non-serious adverse events were recorded at the 6-month visits. All investigators, hospital staff and patients were blinded to treatment at all times and patients received no financial compensation. The study was approved by the research ethics committee of PLA General Hospital as was the statistical analysis of all data. Subjects completed questionnaires capturing nausea, fever and headache status. Skin rash was noted by simple observation. Data on opportunistic infections (HIV, HCV and HBV infections, tuberculosis, gastritis, esophagitis, hypertension, iatrogenic diabetes mellitus, agranulocytosis and osteoporosis) were collected by subjecting blood samples to automated hematological testing using a blood cell counter (model WHY6580; Nanjing Powean Medical Co., Ltd., PR China)[12].

Statistical analysis: All statistical analyses were performed with the aid of SPSS software (ver. 24; SPSS Inc., USA). Demographic and RAS details are reported as numbers and percentages or as means with standard deviations. The paired-samples t-test was used to compare changes in mean ulcer number and size and mean pain intensity, over the study period[21]. The independent-samples t-test was employed to compare differences in means at various timepoints between pairs of arms. The chi-squared test was used to compare the proportions of patients with severe pain and serious and non-serious adverse events, at various timepoints between pairs of arms[14]. Demographic and treatment differences were considered significant at the 99% confidence level at BL and at the 95% confidence level otherwise.

RESULTS

Demographic characteristics (age, sex ratio, body mass index [BMI] and disease features including ulcer number and size and pain severity) were compared among the three treatment arms and in a pairwise manner (Group L vs. Group P, Group P vs. Group L+P and Group L vs. Group L+P). No significant difference was evident at BL ($p \geq 0.01$). The mean ulcer number was significantly reduced at the end of follow-up in all three groups compared with BL ($p \leq 0.05$). Group L+P had the fewest ulcers (compared with Groups P and L) ($p \leq 0.05$). The final ulcer number did not differ significantly between Groups P and L ($p \geq 0.05$). Mean ulcer size was significantly reduced at the 6-month time point in all 3 groups compared with BL ($p \leq 0.05$). During early follow-up, Group L+P had smaller ulcers than Groups P and L ($p \leq 0.05$). However, no significant between-group difference was evident at the 6-month follow-up. The proportion of patients suffering from severe pain at BL was similar in all 3 groups ($p \geq 0.05$). Pain intensity decreased significantly in all 3 groups during follow-up compared with BL ($p \leq 0.05$). At the 6 months follow-up, pain intensity differed significantly among the 3 groups (overall $p \leq 0.05$). Pain was significantly less intense in Group L+P than in Groups P and L ($p < 0.05$ for both comparisons, Table 2). No patient was lost to follow-up.

Table 2: Effects of interventions on ulcer number and size and pain intensity

Time of assessments	Parameters	Group L (n = 100)	Group P (n = 100)	Group L+P (n = 100)	p-value (L vs. P)	p-value (P vs. L+P)	p-value (L vs. L+P)
BL	Number of ulcers	2.6 (1.3)	2.1(1.6)	2.4 (1.9)	0.091	0.656	0.386
3 months		1.8 (1.5)	1.9 (1.3)	1.6 (1.2)	0.615	0.091	0.299
6 months		1.3 (0.9)	1.4 (1.3)	1.1 (0.8)	0.527	0.051	0.098
BL	Size of ulcers (mm)	2.7 (1.2)	2.4 (1.7)	2.8 (1.5)	0.151	0.079	0.603
3 months		2.2 (1.1)	2.1 (1.5)	2.0 (1.2)	0.591	0.603	0.220
6 months		1.9 (1.3)	2.0 (1.1)	1.8 (0.9)	0.557	0.161	0.527
BL	NRS*	53.4 (11.7)	52.7 (9.8)	53.1(9.4)	0.647	0.768	0.841
3 months		42.1(9.8)	41.5 (7.9)	37.9 (2.1)	0.345	0.039	0.041
6 months		35.3 (6.7)	36.3 (5.8)	29.6 (6.3)	0.261	<0.05	<0.05

BL: Baseline, p-values were calculated using the paired samples t-test, Data are shown as Means±SD. *NRS, 101-point Likert numerical rating scale; 0, no pain; 100, worst possible pain

Table 3: Incidence of non-serious treatment-associated adverse events

Adverse events	Group L N (%)	Group P N (%)	p-value (L vs. P)	Group L+P N (%)	p-value (P vs. L+P)	p-value (L vs. L+P)
Agranulocytosis	2 (2)	5 (5)		19 (19)		
HIV infection	0 (0)	1 (1)		3 (3)		
HCV infection	1 (0)	3 (3)		5 (5)		
HBV infection	0 (0)	1 (1)		5 (5)		
Tuberculosis	5 (5)	12 (12)		22 (22)		
Gastritis	15 (15)	19 (19)	≥0.05	35 (35)	≤0.05	≤0.05
Osteoporosis	8 (8)	18 (18)		27 (27)		
Agranulocytosis	2 (2)	5 (5)		9 (9)		
Iatrogenic diabetes mellitus	2 (2)	7 (7)		13 (13)		
Hypertension	7 (7)	11 (11)		18 (18)		
Esophagitis	3 (3)	12 (12)		23 (23)		
Abnormal blood count	3 (3)	7 (7)		24 (24)		

Data are shown as numbers (%), N = 100 for all groups

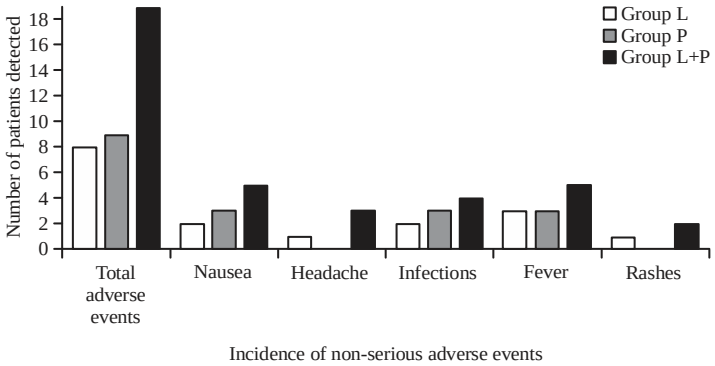

Fig. 3: Incidence of non-serious adverse events, n = 100 for all groups. Adverse events were more prevalent in Group L+P than Group L (p = 0.022) or Group P (p = 0.041) patients

The incidence of non-serious adverse events was higher in Group L+P than in Groups L (p = 0.022) or P (p = 0.041) (Fig. 3). In total, 37 non-serious adverse events were recorded across all three groups, of which fever was the most common (in three patients of each monotherapy group and five combination therapy patients), followed by nausea, headache, infection and rash. No Group P patient developed headache or rash. Overall, the incidence of non-serious adverse events to 6 months was higher in Groups L+P and P compared with Group L (Table 3). However, no serious morbidity was reported.

DISCUSSION

The study performed a well-designed, randomized, prospective, controlled clinical trial. All patients were followed-up to the end of the study and were representative

of typical Chinese patients. Thus, findings are generalizable. Sample size, follow-up time and tested therapies yielded error-free, significant data.

Topical medications are the first-line treatment and systemic medications are the second-line treatment[11]. RAS treatment is not straight forward, requiring several full clinical histories. Treatment must reflect the disease severity (pain), medical history, frequency of ulceration and tolerance to medication[14]. The drugs and route of administration chosen were appropriate for RAS in this study.

To the best of our knowledge, only one prior placebo-controlled study has assessed the effectiveness of combination therapy (levamisole/prednisolone) for patients with RAS. However, the sample size was very small and only levamisole monotherapy was performed[22]. It was concluded that the 2 RAS groups (levamisole alone and the levamisole/prednisolone combination) did not differ significantly in any disease parameter (reduction in ulcer size and number or pain intensity), both treatments were efficacious and the results of the study were similar.

Although patients receiving combination therapy had somewhat fewer ulcers than the other groups at the end of this study, statistical significance was not attained. A previous study comparing groups receiving levamisole alone or a combination therapy reported similar results[22]. However, in terms of ulcer size, the differences between the present study and the earlier work may be attributable to variation in the dosing schedules.

Pain reduction was reported by all patients in the three arms of the present study, this is the major goal of treatment. However, pain intensity differed significantly at the 6-month timepoint. Group L+P patients had significantly less pain than Group P and L patients, attributable to levamisole and prednisolone exerting immunosuppressive and anti-inflammatory actions, respectively. Such dual actions were obviously not available in the monotherapy arms. Several studies found that prednisolone at various doses reduced pain[15,16]. The finding that the addition of a corticosteroid sparing agent reduced pain still further is significant.

The incidence of adverse events was significantly higher in Group L+P than in Groups P and L as might be expected (an additive effect). The results the study were in line with those of a previous study, which reported a similar adverse events profile but with some differences in incidence[22], attributable to the small sample size[23] and differences in patient characteristics[24]. A large sample size is required to reliably document the incidence of adverse events.

The limitations of the study included the non-evaluation of secondary concentration-dependent effects of levamisole/prednisolone combination therapy. furthermore, the study did not record ulcer duration or the frequency of attacks and a larger sample size is needed.

CONCLUSION

Levamisole and prednisolone monotherapy for RAS patients were similar in terms of safety and efficacy. Combination therapy (levamisole and prednisolone) was significantly more efficacious but was associated with more adverse effects then the monotherapies.

SIGNIFICANCE STATEMENTS

This study discovered the efficacy outcomes, fatal and non-fatal treatment emergent adverse events of levamisole and prednisolone as monotherapy and in combination therapy over a 6 months' period in patients with recurrent aphthous stomatitis. This study will help the clinicians to uncover the critical areas of selection of immunomodulators in maxillofacial diseases.

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REFERENCES

1. Sharma, S., F.M. Ali, K. Saraf and A. Mudhol, 2014. Anti-helminthic drugs in recurrent apthous stomatitis: A short review. J. Pharm. Bioallied Sci., 6: 65-68.
2. Scully, C., M. Gorsky and F. Lozada-Nur, 2003. The diagnosis and management of recurrent aphthous stomatitis: A consensus approach. J. Am. Dent. Assoc., 134: 200-207.
3. Field, E.A. and R.B. Allan, 2003. Oral ulceration-aetiopathogenesis, clinical diagnosis and management in the gastrointestinal clinic. Aliment. Pharmacol. Ther., 18: 949-962.
4. Rogers, R.S., 1997. Recurrent aphthous stomatitis: Clinical characteristics and associated systemic disorders. Semin. Cutan. Med. Surg., 16: 278-283.
5. Chattopadhyay, A. and S. Chatterjee, 2007. Risk indicators for recurrent aphthous ulcers among adults in the US. Commun. Dentistry Oral Epidemiol., 35: 152-159.

6. McCullough, M.J., S. Abdel Hafeth and C. Scully, 2007. Recurrent aphthous stomatitis revisited; clinical features, associations and new association with infant feeding practices? J. Oral Pathol. Med., 36: 615-620.
7. Rivera-Hidalgo, F., J.D. Shulman and M.M. Beach, 2004. The association of tobacco and other factors with recurrent aphthous stomatitis in an US adult population. Oral Dis., 10: 335-345.
8. Shulman, J.D., 2004. An exploration of point, annual and lifetime prevalence in characterizing recurrent aphthous stomatitis in USA children and youths. J. Oral Pathol. Med., 33: 558-566.
9. Albanidou-Farmaki, E., A.K. Markopoulos, F. Kalogerakou and D.Z. Antoniades, 2007. Detection, enumeration and characterization of T helper cells secreting type 1 and type 2 cytokines in patients with recurrent aphthous stomatitis. Tohoku J. Exp. Med., 212: 101-105.
10. Guimaraes, A.L., J. de Fatima Correia-Silva, A.R. de Sa, J.M.N. Victoria, M.G. Diniz, F. de Oliveira Costa and R.S. Gomez, 2007. Investigation of functional gene polymorphisms IL-1β, IL-6, IL-10 and TNF-α in individuals with recurrent aphthous stomatitis. Arch. Oral Biol., 52: 268-272.
11. Belenguer-Guallar, I., Y. Jimenez-Soriano and A. Claramunt-Lozano, 2014. Treatment of recurrent aphthous stomatitis. A literature review. J. Clin. Exp. Dent., 6: e168-e174.
12. Weckx, L.L.M., C.H.W. Hirata, M.A.M.M. de Abreu, V.C. Fillizolla and O.M.P. da Silva, 2009. [Levamisole does not prevent lesions of recurrent aphthous stomatitis: A double-blind placebo-controlled clinical trial]. Rev. Assoc. Med. Bras, 55: 132-138.
13. Sun, A., J.T. Wang, J.S. Chia and C.P. Chiang, 2006. Levamisole can modulate the serum tumor necrosis factor-α level in patients with recurrent aphthous ulcerations. J. Oral Pathol. Med., 35: 111-116.
14. De Abreu, M.A.M.M., C.H.W. Hirata, D.R.N. Pimentel and L.L.M. Weckx, 2009. Treatment of recurrent aphthous stomatitis with clofazimine. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endodontol., 108: 714-721.
15. Femiano, F., C. Buonaiuto, F. Gombos, A. Lanza and N. Cirillo, 2010. Pilot study on Recurrent Aphthous Stomatitis (RAS): A randomized placebo-controlled trial for the comparative therapeutic effects of systemic prednisone and systemic montelukast in subjects unresponsive to topical therapy. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endodontol., 109: 402-407.
16. Pakfetrat, A., A. Mansourian, F. Momen-Heravi, Z. Delavarian, J. Momen-Beitollahi, O. Khalilzadeh and S. Basir-Shabestari, 2010. Comparison of colchicine versus prednisolone in recurrent aphthous stomatitis: A double-blind randomized clinical trial. Clin. Invest. Med., 33: E189-E195.
17. Brocklehurst, P., M. Tickle, A.M. Glenny, M.A. Lewis and M.N. Pemberton *et al*., 2012. Systemic interventions for recurrent aphthous stomatitis (mouth ulcers). Cochrane Database Syst. Rev. 10.1002/14651858.CD005411.pub2.
18. Cui, R.Z., A.J. Bruce and R.S. Rogers, 2016. Recurrent aphthous stomatitis. Clin. Dermatol., 34: 475-481.
19. Karlberg, J.P.E. and M.A. Speers, 2010. Reviewing Clinical Trials: A Guide for the Ethics Committee. Clinical Trials Centre, Washington, DC., ISBN: 9789881904119, Pages: 153.
20. Charan, J. and T. Biswas, 2013. How to calculate sample size for different study designs in medical research? Indian J. Psychol. Med., 35: 121-126.
21. Rezende-Neto, J., T. Rice, E.S. Abreu, O. Rotstein and S. Rizoli, 2016. Anatomical, physiological and logistical indications for the open abdomen: A proposal for a new classification system. World J. Emergency Surg., Vol. 11. 10.1186/s13017-016-0083-4.
22. Sharda, N., M.C. Shashikanth, P. Kant and M. Jain, 2014. Levamisole and low-dose prednisolone in the treatment of reccurent aphthous stomatitis. J. Oral Pathol. Med., 43: 309-316.
23. Chan, Y.H., 2003. Randomised controlled trials (RCTs)-Sample size: The magic number? Singapore Med. J., 44: 172-174.
24. Showstack, J., P.P. Katz, J.R. Lake, R.S. Brown Jr. and R.A. Dudley *et al*., 1999. Resource utilization in liver transplantation: Effects of patient characteristics and clinical practice. J. Am. Med. Assco., 281: 1381-1386.