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Systematic Review

Outcomes of surgical interventions for the treatment of limbal stem cell deficiency

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***Background & objectives*: In the current scenario, with availability of different surgical procedures for limbal stem cell deficiency (LSCD), there exists no common consensus as to the standardization of the management protocol for the same. In addition, there also exists diversity in the views about the clinical diagnosis, ancillary investigations and clinical parameters. The objective of the present study was to evaluate the reported outcomes of surgical interventions for the management of LSCD.**

***Methods*: A systematic review of published literature on limbal stem cell transplantation (LSCT) was performed using Ovid Medline, Embase and PubMed for a duration of 2009 to 2019. Original studies including prospective, retrospective case series and randomized controlled trials, articles in English language, articles with access to full text and studies with more than or at least 10 patients were included in this review. Data related to clinical and visual outcomes were evaluated, and pool estimates of different surgeries were calculated using random-effects model and individually using Pearson's Chi-square test.**

***Results*: A total of 1133 abstracts were evaluated. Finally, 17 studies were included for the analysis. Among these 17 studies, direct limbal lenticule transplantation was performed in five studies, of which autologous tissue from the fellow eye [conjunctival limbal autograft (CLAU)], allograft from a cadaver/live donor [keratolimbal allograft (KLAL)/conjunctival limbal allograft (CLAL)] and combination of CLAU plus KLAL were done in one, three and one studies, respectively. The *ex vivo* expanded cultivated limbal epithelial transplantation (CLET) was reported in six studies and simple limbal epithelial transplantation (SLET) in four studies. Two were comparative studies comparing CLET and CLAL (living-related CLAL) with cadaveric KLAL, respectively. Outcome analysis of the included studies showed significant heterogeneity. Calculated pool rate for various types of surgeries was calculated. The pool estimate for CLAL was 67.56 per cent [95% confidence interval (CI), 41.75-93.36; I^2=83.5%, *P*=0.002]. For KLAL, this value was 63.65 per cent (95% CI, 31.38-95.91; I^2=92.4%, *P*=0.000). Pool estimate for CLET was 78.90 per cent (95% CI, 70.51-87.28; I^2=73.6%, *P*=0.001). Corresponding values for SLET were 79.08 per cent (95% CI, 74.10-84.07; I^2=0.0%, *P*=0.619). CLAU and combination of CLAU plus KLAL were done in one study each; hence, statistical analysis could not be done. The functional outcome in terms of gain in visual acuity post-operatively was better in KLAL (*P*<0.005) and SLET group as compared to CLET group.**

***Interpretation & conclusions*: The present analysis suggests that though the anatomical success rates were almost identical between SLET, CLET, CLAL, and KLAL procedures, the functional success rates were better following KLAL and SLET procedures as compared to CLET. Decision for LSCT for cases of ocular burns based on either clinical judgement of the surgeon or individual diagnosis remains a suitable option.**

Key words Chemical injury - cornea - limbal stem cell deficiency - limbus - ocular burns - ocular surface - transplantation

Limbal stem cells are essential for maintaining corneal transparency, as these cells constantly replenish damaged corneal epithelial cells[1]. Various factors can be attributed to limbal stem cell damage. These include physical, chemical, thermal and immunological insults, of which chemical injury contributes a major proportion. Limbal stem cell deficiency (LSCD) manifests in the form of chronic ocular surface inflammation with persistent epithelial defects, corneal vascularization and conjunctivalization eventually, leading to visual disturbance[1]. Management of ocular stem cell deficiency has slowly gained momentum with emerging techniques and surgical interventions[2]. Over the past several years, evidence-based research has suggested a potential role of diverse surgical modalities for replenishment and restoration of the architecture of the limbal stem cells and ocular surface. The treatment modalities have gradually evolved to the present surgical techniques to address the problems of LSCD and provide better outcomes.

The choice of procedure for LSCD depends on the extent of the ocular surface involvement (partial vs. total), the laterality (unilateral or bilateral), absence or presence of ongoing inflammation or infection and associated secondary glaucoma. Partial LSCD can be treated by either denuding cornea at visual axis or resurfacing the cells from the unaffected healthy limbal epithelium[3-6]. However, in cases with total LSCD, either autologous limbal lenticule from the fellow eye or allograft from the cadaver/live-related donors is required[7-17]. However, with the availability of different surgical procedures, there lies no common consensus as to the standardization of the management protocol for cases with LSCD. There also exists diversity in the views about the clinical diagnosis, ancillary investigations and clinical parameters. Conjunctivalization, vascularization and loss of transparency are the significant parameters used to establish the diagnosis. Impression cytology and ocular surface staining are among the ancillary investigations described in the past[18].

In the present systematic review, surgical interventions including direct transplantation of the limbal lenticule either as an autologous tissue from the fellow eye [conjunctival limbal autograft (CLAU)][19] and a keratolimbal allograft (KLAL) from a cadaver and from live donor [conjunctival limbal allograft (CLAL)][20-24] or the combination of the[25] two were evaluated. In addition to direct transplantation of limbal lenticule, *ex vivo* expanded cultivated limbal epithelial transplantation (CLET)[26-31] and simple limbal epithelial transplantation (SLET)[32-35] were evaluated, and the results of these surgical interventions were discussed. In the present review, though cultivated oral mucosal epithelial transplantation (COMET) studies have been described as this procedure has been reported to promote re-epithelialization and helped in reducing inflammation in patients with acute LSCD[36-40], COMET studies have not been included in the analysis.

The objective of this systematic review was to evaluate and compare the efficacy/outcomes of different surgical interventions for LSCD. This was done to analyze and strengthen evidence to facilitate the adoption of best clinical and surgical practices for the management of LSCD.

Material & Methods

The study was conducted in accordance with the PRISMA guidelines for systematic review[41]. Documentation of data was done following a set protocol before commencement of literature search.

Outcome measures: The primary objective was to determine the best acceptable practices for surgical management of LSCD. Data related to surgical outcomes were evaluated in terms of anatomical success (absence of persistent epithelial defect, vascularization and conjunctivalization) and functional success (improvement in visual acuity).

Methods followed for study selection: Electronic literature search using Ovid Medline, Embase and PubMed was performed for studies published from

2009 to 2018 using limbal stem cell, limbal stem cell deficiency, chemical injury or ocular burns, conjunctival limbal autograft transplantation, cultivated limbal epithelial transplantation, simple limbal epithelial transplantation, cultured ocular mucosal epithelial transplantation and limbal stem cell transplant as keywords. Search was further supplemented by retrieving cross-references. Studies which were published before 2009 were excluded from this analysis, as Cauchi *et al*[42] published a review article on related literature till 2008. Recently, Shanbhag *et al*[43] published a systematic review on outcomes of three different techniques of autologous limbal stem cell transplantation (LSCT) in unilateral LSCD.

Eligibility criteria: Original studies including prospective, retrospective case series and randomized controlled trials, articles in English language, those with access to full text and those with at least 10 or more patients were included in this review. Experimental, animal studies and correspondences letters were excluded.

A total of 1133 abstracts were identified through database search and 15 additional studies were found to be relevant after checking cross-references. Of the total 1148 articles, 21 records were selected for analysis after removing duplicate studies and studies not directly related to the review topic. Of these, four more articles were excluded, reason being animal/experimental studies and case reports with one or two patients only. Finally, 17 studies were incorporated in the final analysis for quantitative and qualitative assessment (Fig. 1).

Interventions: Various surgical modalities of LSCT such as CLAU, CLAL, KLAL, CLET, COMET and SLET were evaluated.

Study selection & data extraction: Three authors have done eligibility assessment independently in an unblended standardized manner. Wherever full manuscript was available, it was carefully reviewed by all the three reviewers. Any disagreement between the three was resolved by unanimous consensus. While extracting data, relevant parameters, *i.e.* author's name, name of the journal, year of publication, type of study design and various factors, that can affect outcomes were noted carefully**.**

Assessment of methodological quality of the selected studies: As per the PRISMA guidelines, methodological quality of each component study was evaluated. Score was given depending upon the answers to certain questions, such as, whether operating surgeon concealed to the reviewer of outcomes or not, whether dropouts were <15 per cent or not, whether follow up intervals were pre-specified or patients' charts were just reviewed and whether detailed description of outcomes were there or not[41]. Scoring was done as 2, 1 and 0 for clearly affirmative, probably affirmative or clearly negative answer, respectively.

Statistical analysis: Stata 15.0 (StataCorp LLC, College Station, TX 77845, USA) statistical software was used for analysis. The pooled estimates [95% confidence interval (CI)] for anatomical success after LSCT (absence of persistent epithelial defect, vascularization and conjunctivalization)[1] were calculated using random-effects model for CLAL, CLET, KLAL and SLET procedures. Random-effects model was used to pool the various studies results as per the type of surgical interventions. Outcome analysis of the included studies was evaluated by calculating pool estimate value with 95 per cent CI. Data from all the included studies were divided and subdivided depending upon the type of intervention, *i.e.* CLAL, KLAl, CLET and SLET. However, CLAU and combination of CLAU plus KLAL were done in one study each; hence, the pooled estimate could not be calculated for these two. However, anatomical success after LSCT (absence of persistent epithelial defect, vascularization and conjunctivalization) and functional success (improvement in visual acuity) were compared between various surgical groups using Pearson's Chi-square test.

Results

The surgical outcomes in terms of anatomical and functional (visual) success were assessed in details for seventeen eligible studies[19-35].

Study quality: All 17 studies (100%) achieved adequate follow up and had mentioned detailed description of outcome criteria, dropouts were <15 per cent and pre-specified and follow up parameters were mentioned. The quality score ranged from 6 to 7 points with mean and median value of 6.05 and 6, respectively.

Owing to statistical heterogeneity, the outcomes of different type of surgeries were analyzed separately by calculating pooled estimate. Hence, the outcomes of surgical interventions are summarized in a descriptive manner as well. The demographic and descriptive characteristics of the included studies are shown in the Table.

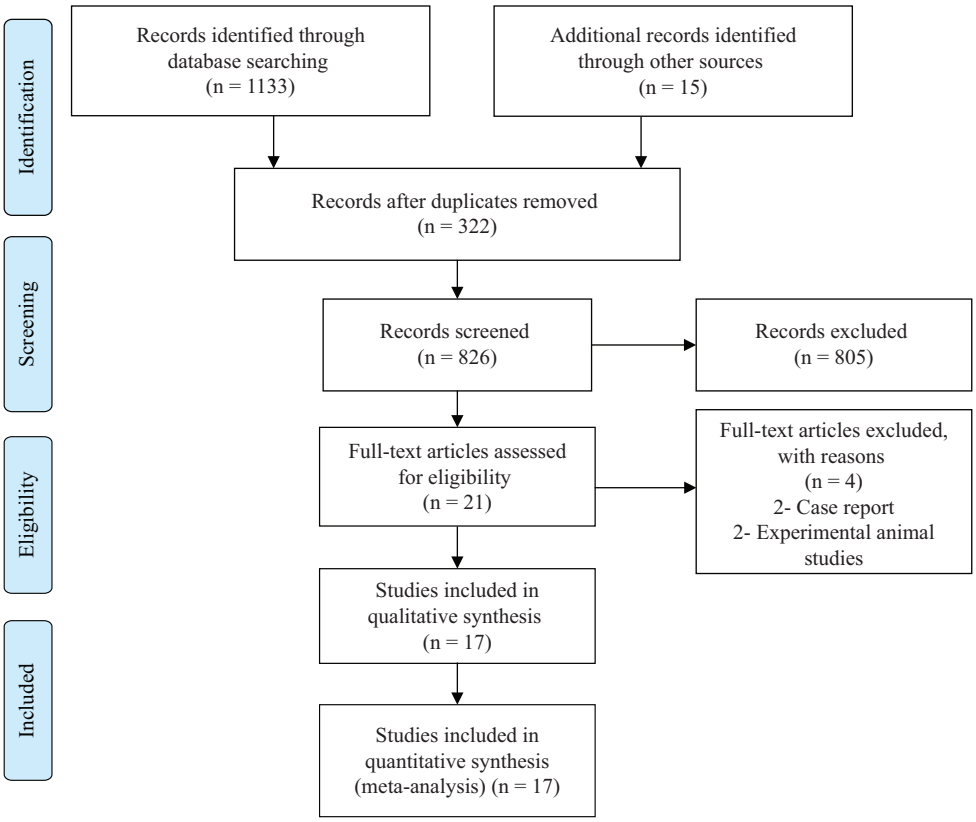

Fig. 1. Flowchart representing study selection process as per the PRISMA guidelines.

Calculated pool rate for various types of surgeries was calculated. The pool estimate for CLAL was 67.56 per cent (95% CI, 41.75-93.36; I^2=83.5%, P=0.002) as depicted in Fig. 2. For KLAL, the values were 63.65 per cent (95% CI, 31.38-95.91; I^2=92.4%, P=0.001) as depicted in Fig. 3. Pool estimate for CLET was 78.90 per cent (95% CI, 70.51-87.28; I^2=73.6%, P=0.001) as depicted in Fig. 4. Corresponding values for SLET were 79.08 per cent (95% CI, 74.10-84.07; I^2=0.0%, P=0.619) as depicted in Fig. 5. CLAU and combination of CLAU plus KLAL were done in one study each; hence, statistical analysis could not be done.

Of the 17 studies, 10 were retrospective and nine were prospective. Among the analyzed studies, direct transplantation of the limbal lenticule either as an autologous tissue from the fellow eye (CLAU) or as an allograft from a cadaver/live donor (KLAL/CLAL) and combination of CLAU plus KLAL were done in one, three and one studies, respectively. The *ex vivo* expanded cultivated limbal epithelial transplantation (CLET) and SLET were done in six and four studies, respectively. One study each compared CLAL versus KLAL and CLET versus KLAL.

CLAU, CLAL and KLAL surgical outcomes: Direct limbal lenticule was transplanted in five studies[19-22,25], and one was a comparative study between CLAL and KLAL[25]. Transplantation of autologous lenticule tissue from the fellow eye (CLAU) was done in a study by Baradaran-Rafii *et al*[19], an allograft from a live-related donor (CLAL) in study by Barreiro *et al*[20] and Scocco *et al*[21], an allograft from cadaver (KLAL) in a study by Baradaran-Rafii *et al*[22] and combined CLAU plus KLAL intervention by Chan *et al*[25].

Baradaran-Rafii *et al*[19] evaluated surgical outcomes of CLAU in 34 patients having unilateral LSCD. Chemical injury was attributed as a cause of LSCD in 73.5 per cent of patients. The authors reported anatomical success, visual success and failure in 88, 88 and 11.8 per cent of the patients, respectively. Complications such as conjunctival encroachment on to the graft, graft dislodging due to small size, thick graft, graft oedema, ocular surface exposure, progressive horizontal conjunctivalization and pyogenic granuloma were reported during the post-operative period[19].

Similar studies done by Barreiro *et al*[20] and Scocco *et al*[21] looked at surgical outcomes of CLAL in 34

Table. Descriptive presentation of the various studies done to evaluate and compare different techniques of limbal stem cell transplantation

Authors	Eye laterality	Type of study	Intervention	Number of patients	Mean age (yr)	Mean follow up (months)	MC primary pathology	Number of patients with MC primary pathology (n)	Primary success (%)	Secondary success (%)	Failure (%)
Baradaran-Rafii *et al*[19]	U/L LSCD	Retrospective	CLAU	34	27.3	17.2	Chemical injury	25	88	88	11.8
Barreiro *et al*[20]	U/L+B/L	Retrospective	CLAL (live related)	34	37.3	18.9	Chemical	34	47.4	82.1	52.6
Scocco *et al*[21]	B/L	Retrospective	CLAL (live related)	32	33.62	48.7	SJS	14	84.6	46.2	15.4
Baradaran-Rafii *et al*[22]	B/L	Retrospective	KLAL	45	26.7	26.1	Chemical	36	73.4	84.8	26.6
Chan *et al*[25]	U/L	Prospective, interventional case series	CLAU + KLAL	11	31.6	35.8	Chemical	6	82	73	18
Vazirani *et al*[26]	U/L	Retrospective	CLET	70	24	17.5	Chemical	28	75	66.6	25
Sangwan *et al*[27]	U/L	Retrospective	CLET	200	24.1	36	Chemical	162	71	60.5	29
Prabhasawat *et al*[28]	U/L+B/L	Prospective	CLET	18	44.7	26.1	Chemical	13	77.9	73.7	26.3
Rama *et al*[29]	U/L+B/L	Prospective	CLET	112	46.5	34.9	Chemical	109	89.7	41.07	10.3
Shortt *et al*[30]	U/L+B/L	Prospective	CLET	10	46.1	13	Chemical	4	60	60	40
Ramírez *et al*[31]	U/L+B/L	Prospective	CLET	19	51.6	36	Non-IF cause*	9	80	50	20
Vazirani *et al*[32]	U/L	Retrospective	SLET	68	22	12	Chemical	62	83.8	64.7	16.2
Basu *et al*[33]	U/L	Prospective	SLET	125	62.5	18	Chemical	125	76	75.2	18.4
Basu *et al*[34]	U/L	Prospective case series	SLET	30	15	27.6	Chemical	30	80	62.5	23.3
Gupta *et al*[35]	U/L	Prospective case series	SLET	30	G1:29.1 G2:9.1	12 12	Chemical	38.8 83.3	70 83.3	71.4 71.4	30 16.7
Comparative, prospective interventional studies											
Titiyal *et al*[23]	U/L	Prospective	CLAL (G1) versus KLAL (G2)	20	G1:18.1 G2:17	6	Chemical/ thermal burns	20	G1:70 G2:20	G1:80 G2:40	G1:30 G2:80
Parihar *et al*[24]	B/L	Prospective	CLET (G1) versus KLAL (G2)	40 50 eyes	G1:46 G2:48	12	Chemical/ thermal burns	32	G1:86.96 G2:91.67	G1:76 G2:72	G1:NA G2:NA

U/L, unilateral; B/L, bilateral; CLAU, conjunctival limbal autograft *i.e.,* direct transplantation of the limbal tissue lenticule (autologous tissue); KLAL/CLAL, keratolimbal allograft/conjunctival limbal allograft *i.e.,* allograft from a cadaver/live donor; CLET, *ex vivo* expanded cultivated limbal epithelial transplantation; SLET, simple limbal epithelial transplantation; COMET, cultivated oral mucosal epithelial transplantation; SJS, Steven-Johnson syndrome; IF, inflammatory; G1, group 1; G2, group 2; LSCD, limbal stem cell deficiency; MC, multiple sclerosis

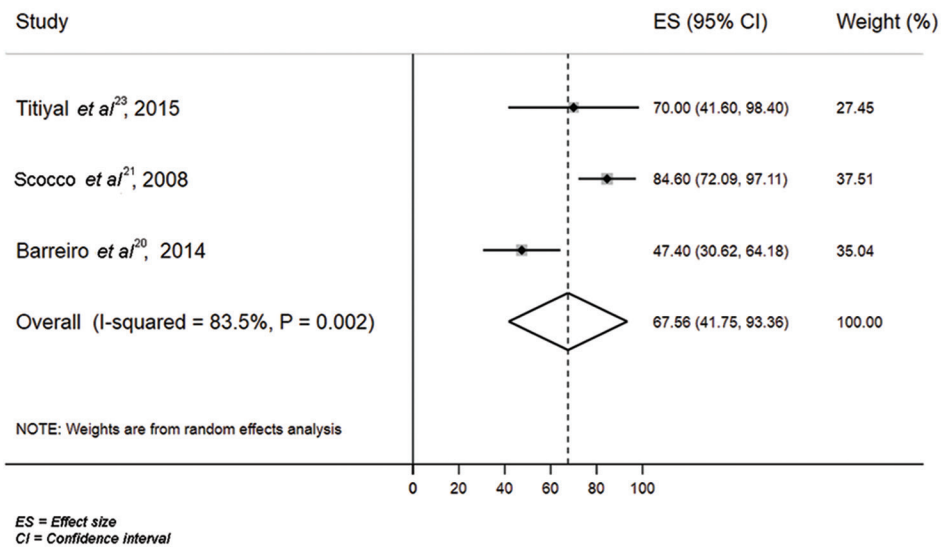

Fig. 2. Clinical outcome pooled estimate for conjunctival limbal allograft surgery.

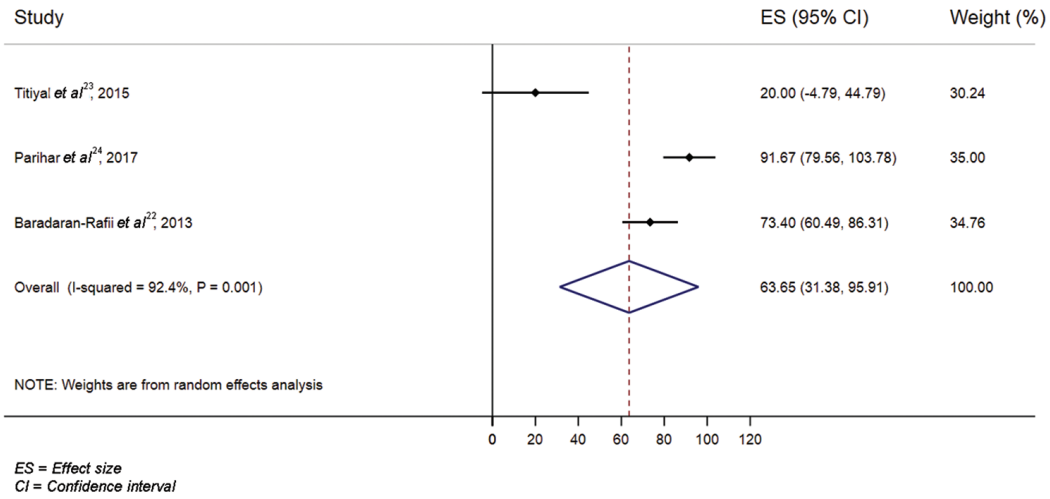

Fig. 3. Clinical outcome pooled estimate for keratolimbal allograft surgery.

and 32 patients, respectively. Barreiro *et al*[20] included patients with unilateral and bilateral LSCD, and Scocco *et al*[21] enrolled patients with bilateral LSCD alone. The major cause of LSCD in these studies was chemical injury (100%)[20] and Steven–Johnson syndrome (43.7%)[21], respectively. These two studies reported anatomical success, visual success and failure in 47.4, 82.1 and 52.6 per cent and 84.6, 46.2 and 15.4 per cent of the patients, respectively[20,21]. Along with the assessment of surgical outcomes of CLAL, Barreirio *et al*[20] compared the outcomes of conjunctival limbal transplantation with and without the use of amniotic membrane (AM) transplantation and documented similar results with both the procedures. In another study, no immunosuppressants were given and the limbal graft rejection was reported in 17.9 per cent of the cases[21], whereas in study by the Barreiro *et al*[20], immunosuppression was given in HLA-incompatible cases.

Baradaran-Rafii *et al*[22] used cadaveric limbal allografts (KLAL) for transplantation in 45 patients with bilateral LSCD. Similar to the previously cited study[21], chemical injury was the most common cause of LSCD (80% cases) and authors noted anatomical success, visual success and failure in 73.4, 84.8 and 26.6 per cent of patients, respectively[22]. In addition, adverse events such as immunologic rejections, graft-related

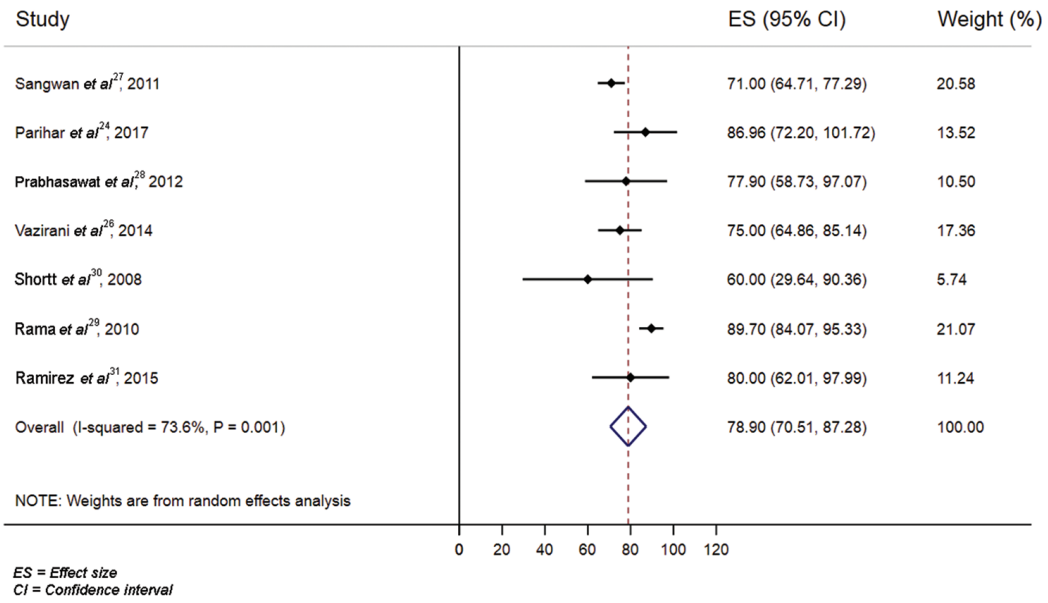

Fig. 4. Clinical outcome pooled estimate for cultivated limbal epithelial transplantation surgery.

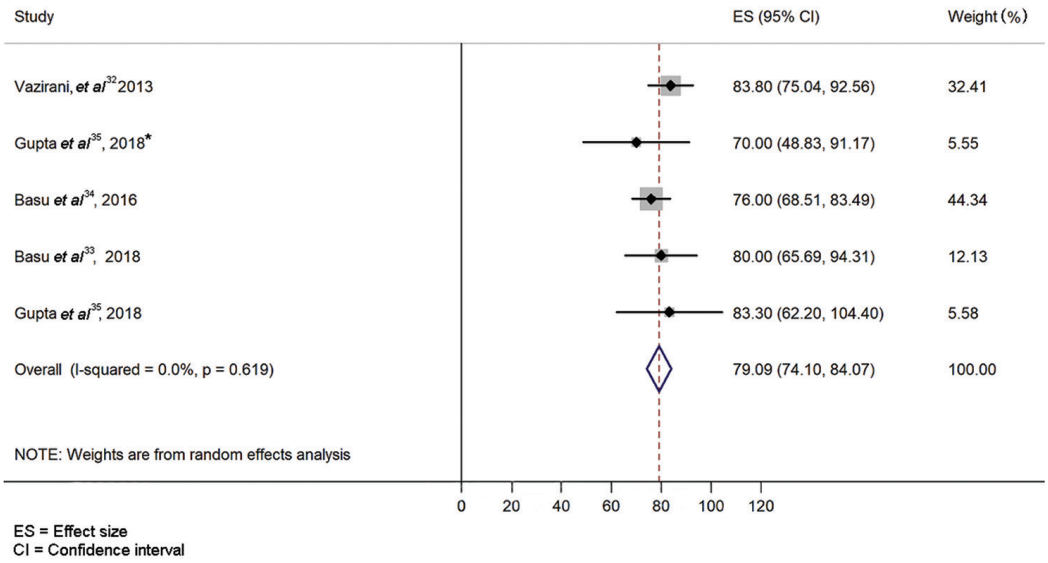

Fig. 5. Clinical outcome pooled estimate for simple limbal epithelial transplantation surgery.

complications and chronic ocular surface exposure were reported post-KLAL surgery. Chan *et al*[25] evaluated outcomes of combined procedure of CLAU and KLAL. They included 11 patients with unilateral LSCD (attributed mainly to chemical injury, *i.e.* 54.5% patients) and documented anatomical success, visual success and failure in 82, 73 and 18 per cent of patients, respectively. Titiyal *et al*[23] compared living-related CLAL with KLAL in a prospective interventional study. The study included 20 patients with unilateral LSCD (attributed to ocular burns) divided randomly into the two groups. At six months of follow up, the eyes that underwent CLAL as compared to those which underwent KLAL did significantly better in terms of visual gain (80 vs. 50%), ocular surface stability (70 vs. 20%), regression of conjunctivalization and corneal neovascularization. No complications were noted in either of the groups at the end of six months[23].

Ex vivo expanded CLET: CLET was performed in six studies, of which two analyzed outcomes in patients with unilateral LSCD and four studies analyzed patients with

both unilateral and bilateral LSCD[26-31]. Vazirani *et al*[26] looked at surgical outcomes of CLET in 70 patients with unilateral LSCD (40% post-chemical injury) and reported anatomical success, visual success and failure in 75, 66.6 and 25 per cent, respectively. This study compared the outcomes of autologous CLET also, either using the healthy part of the affected eye or the fellow eye as a source of limbal stem cells. The authors concluded that outcomes were similar and irrespective of the location of donor area[26].

Sangwan *et al*[27] evaluated post-CLET data of 200 patients with unilateral LSCD (81% post-chemical injury) and documented anatomical success, visual success and failure in 71, 60.5 and 29 per cent of patients, respectively. However, Prabhasawat *et al*[28] and Rama *et al*[29] reported anatomical success, visual success and failure in 77.9, 73.7, 26.3 per cent of patients and 89.7, 41.1 and 10.3 per cent of patients, respectively, of the total 18 and 112 patients with both unilateral and bilateral LSCD, respectively. The main aetiology for LSCD in these two studies noted was chemical injury; in 40 and 97.3 per cent of patients, respectively.

Shortt *et al*[30] looked at surgical outcomes in 10 patients with both unilateral and bilateral LSCD and found anatomical success, visual success and failure in 60, 60 and 40 per cent of patients, respectively. Ramírez *et al*[31] reported anatomical success, visual success and failure in 80, 50 and 20 per cent of patients, respectively, of a total of 19 patients with both unilateral and bilateral LSCD. In the study by Shortt *et al*[30], chemical injury was the main cause of LSCD (40%), whereas in the study by Ramirez *et al*[31], non-inflammatory conditions contributed maximally as a cause for LSCD (47.4%).

A prospective interventional study was conducted by Parihar *et al*[24] which compared CLET with KLAL in 50 eyes of the 40 patients with bilateral LSCD. The most common indication for LSCD in their study was chemical and thermal burns (64%). They concluded that, at one year follow up, both the techniques were comparable. The complications reported included persistent epithelial defect, infective keratitis and primary graft rejection[24].

Simple limbal epithelial transplant: There were four studies that evaluated the outcomes of SLET in cases of unilateral LSCD. Vazirani *et al*[32] retrospectively evaluated and described the outcomes in 68 patients following SLET. The median follow up being 12 months, clinical success rate was 83.3 and in 65.7 per cent cases significant visual improvement was documented. Further supporting this study, a prospective interventional study by Basu *et al*[33] suggested a success rate of 76 per cent and a failure rate of 18.4 per cent in post-SLET procedure. The median follow up was 18 months. Basu *et al*[33] also supported the clinical success with histopathological evidence. The immunohistochemistry of the corneas post-SLET procedure was suggestive of corneal phenotype comparable to normal corneas. Basu *et al*[34] evaluated the outcomes of SLET in failed CLET cases and showed successful outcomes in 80 per cent patients, observed over the period of 2.3 yr (mean follow up). Gupta *et al*[35] published results of SLET in unilateral ocular surface burns in 30 patients. This study reported clinical success in 70 per cent patients. Visual acuity gain was seen in 71.4 per cent of cases. Conclusion of the study mentioned that autologous SLET was an effective limbal cell transplantation technique for the treatment of unilateral LSCD[35]. These studies revealed that following SLET, donor eye suffers minimal damage, the most common observation being a sub-conjunctival haemorrhage.

Cultivated oral mucosal epithelial transplantation: COMET promotes re-epithelialization and helps in reducing inflammation in patients with acute LSCD[36-39]. However, analysis of COMET studies was not performed as it is not purely an LSCT procedure. Sotozono *et al*[36] reported the effectiveness and safety of COMET. This study concluded that substantial visual improvement was noted in patients with end-stage severe ocular surface disorders[36]. Hirayama *et al*[37] compared the clinical results of COMET of substrate-free sheets with those of COMET with AM-based sheets and reported better outcomes with COMET of a substrate-free cell sheet in comparison to COMET with AM. Satake *et al*[38] evaluated the long-term outcome of COMET for treatment of eyes with total LSCD and concluded that this surgery offered a viable and safe alternative in the reconstruction of a stable ocular surface with the additional advantage of a lower incidence of complications. Prabhasawat *et al*[39] evaluated the outcomes of autologous COMET on human AM in 18 patients with bilateral severe LSCD. The authors concluded that COMET was instrumental in successful restoration of the ocular surface in majority of LSCD patients[39].

Comparison of primary and secondary measures CLAL vs. CLET vs. KLAL vs. SLET: Overall, for the

primary outcome (maintenance of a stable, epithelized, avascular cornea), there was no significant difference between any of the surgical procedures. However, the functional outcome in terms of gain in visual acuity post-operatively was better in KLAL group as compared to the CLET group (P=0.005) and SLET group as compared to CLET group (P<0.001). Hence, the anatomical success rates were almost identical between SLET, CLET, CLAL and KLAL procedures, while the functional success rates were better following KLAL and SLET procedures.

Discussion

The present review highlights that although a variety of surgical modalities for the management of LSCD exists, yet there is a lack of standard protocol for deciding a particular intervention over the other. Hence, this analysis was aimed to emphasize on the deficiency in the previous studies designs, including the use of heterogeneous study parameters, and to critically evaluate them. Further studies are needed to compare these surgical modalities in terms of their availability, efficacy, cost factor, technical difficulties and long-term results.

In the previous studies, surgical interventions were done at dissimilar stages of LSCD. Since the severity and stage of ocular surface disease were not comparable at the time of intervention, outcomes might be variable and non-comparable. This also suggests that the stage of LSCD at the time of intervention may be an important deciding factor for choosing any intervention. In the studies included, exact extent (in terms of clock hours) of LSCD was not mentioned, though division into partial and total LSCD was documented in a few studies. Pre-operative extent of LSCD is an important confounding factor for the surgical outcomes, which has not been taken into consideration in majority of the studies.

The percentage contribution of underlying aetiology to LSCD also varied between different studies, which could be another confounding factor while assessing surgical outcomes. Of the total 17 studies, ocular burn (chemical/thermal) was the main cause of LSCD in most of the studies. The response of ocular surface in terms of post-operative inflammation and rate of epithelization may vary depending upon the underlying cause for ocular LSCD.

Another important confounding factor was the wide variation in the number of previous surgical interventions before LSCT. In all 17 studies analyzed, the number of previous surgeries done in acute stage of the ocular insult varied considerably, which may be responsible for variable surgical outcomes even with the same surgical procedure. The success rates tend to drop with increasing number of surgical procedures. In the present review, no randomized controlled trial was found. All the studies were either prospective or retrospective with large variations in sample size, resulting in heterogeneity.

Among CLAU, CLAL, and KLAL studies, surgical outcomes of CLAU reported by Baradaran-Rafii *et al*[22] was better than CLAL and KLAL. However, demographic details of all these studies were not comparable. Additional benefit mentioned for CLAU in this study was the non-requirement of systemic immunosuppression. Immunological rejection contributes significantly to post-operative complications following CLAL and KLAL, more so with KLAL. Thus, systemic immunosuppression becomes important which has its own adverse effects. It is known to decrease the systemic immunity and pre-dispose to infections and requires constant monitoring of the systemic status. Attributing to these reasons, this procedure is not popular despite acceptable surgical outcomes.

In CLET studies, documented surgical outcomes were nearly comparable between the studies and better than that of direct limbal lenticule transplant surgeries. Cultivation of limbal stem cells allows retrieving a smaller sample of limbal tissue from the donor eye as compared to the tissue size required for a direct transplant. The CLET procedure requires association with extensive and specialized clinical laboratory for cultivation of limbal stem cells retrieved from the donor eye. This, in turn, raises issues regarding the cost-effectiveness of CLET and occurrence of LSCD in the donor eye due to the procedure.

SLET has recently emerged as an effective modality for LSCD reported to have a favourable outcome in most studies[43]. The findings of this systematic review suggest that SLET and KLAL have significantly better functional success rates compared with CLET. The advantage of this procedure is that it requires harvesting of a smaller section of limbal tissue from donor eye and that there is no need for clinical laboratory and systemic immunosuppression. SLET has slowly gained popularity due to its advantage of being a single staged hassle-free treatment in

unilateral LSCD. Limitation being bilateral cases, where other modalities such as CLAL or KLAL may be useful.

Although substantial evidence is lacking to support any one particular surgical technique, but studies are available which, despite their heterogeneity, allow assessment of results of different procedures for LSCT in terms of anatomical and functional outcome. This information along with clinical experience of the surgeon can help decide the appropriate intervention based on individual indications.

Future studies may be done taking into account these factors and then comparing the various procedures after standardizing factors, such as baseline inflammatory status of ocular surface, cause of LSCD, stage of LSCD and clock hours of involvement. Systematic data collection can help in better understanding of the outcomes based on stage of disease. Further, it can help formulate standardized intervention methodology for different stages of LSCD to ensure uniformity in outcomes.

Limitations of the review: Regulatory issues in different settings, varied age groups of recruited patients, different regions, difference in type, stage, severity of LSCD, duration between injury and surgery, different culture methods and difference in primary and subsequent intervention were confounding factors. Variable study designs of included studies and lack of any randomized controlled trial contributed to the lack of any concrete inference on this subject. Further studies with standardized methods and homogenous study design and patient population will help in filling the existing lacunae in the literature.

Conclusion

There is some evidence to suggest that although the anatomical success rates were almost identical between SLET, CLET, CLAL and KLAL procedures, yet the functional success rates were better following KLAL and SLET procedures as compared to CLET. Clinical judgement and experience of the surgeon is the underlying parameter for choosing a suitable intervention by considering patient factors and availability of resources.

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