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REVIEW ARTICLE

Human Amniotic Membrane Usage from Bench to Bedside in Congenital Defects and Wounds in Paediatric Surgery: A Systematic Review

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ABSTRACT:

Background: The restricted donor area in paediatric patients demands the use of Human Amniotic membrane (hAM) in the management of difficult-to-manage wounds. It can be used directly over wounds or used to grow stem cells by different culture methods. The hAM can be used "fresh" i.e., +4O glycerol preserved or "cryopreserved".

Methods: In this literature review, we searched 'PubMed', 'Web of Science' and 'SCOPUS' for experimental models, RCT, Observational studies, case series, and case reports involving the usage of hAM in the treatment of neonatal and paediatric (<14 yrs.) patients, published in from 1993 to April 2022. The search included the keywords, "amnion", "biomaterials", "biological dressing", "clinical study", "congenital defects", "human amniotic membrane", "paediatric wound", "neonatal wounds", and "regenerative medicine". The Search was extended by snowballing the reference list of all included studies.

Results: The final analysis included one experimental RCT, three review articles, and twelve case reports. The experimental studies were in rat, pup, and porcine models. The paediatric second-and third-degree thermal burn followed by paediatric ocular diseases viz corneal epithelial ulcers, conjunctiva reconstruction following Steven Johnson Syndrome, and scarring after surgery of strabismus were the most common indications for the usage of AM. Five cases of meningomyelocele repair (intradural & extradural placement of AM) and 2 cases of gastroschisis repair (as an antiadhesive layer) were reported. Freeze-dried hAM is most frequently used in clinical practice. Autologous hAM was used in antenatally detected birth defects. In the adults, the fresh hAM was found equally effective as freeze-dried AM, but with the risk of transmission of contagious diseases. The literature on Fresh amnion is deficient in paediatric patients.

Conclusion: hAM as a skin substitute in paediatric wounds/defects has shown an enhanced rate of healing. However, further studies, regarding the utility of hAM in the management of paediatric wounds, congenital anatomical defects, and diseases along with analysis of outcome and economic constraints in developing countries are needed

Keywords: amnion, biomaterials, biological dressing, clinical study, human amniotic membrane, paediatric wound management, neonatal wounds, neonatal necrotizing fasciitis, paediatric burn wound, regenerative medicine.

Background: Besides anatomical defects of the anterior abdominal wall and Spina Bifida Aperta, thermal burn, necrotizing fasciitis, Toxic epidermolysis bullosa, Steven-Johnson syndrome, and rarely cutis aplasia involve a wide area of skin surface damage in neonates and children1,2. The neonates and children have limited body surface area, peculiar distribution of donor surface areas, less subcutaneous tissue, and less tensile strength in the skin. Thus, harvesting skin grafts in neonates and children is difficult3. Further, the harvesting of skin grafts is not possible in infants because the skin is immature having less tensile strength2. Sometimes functional resurfacing of skin could not be achieved by primary suturing or delayed primary suturing. Traditionally, wound care in these patients is done with healing by secondary intention4. The disadvantages of wound healing by secondary intention are long healing time, risk of secondary infection, wound contracture, increased morbidity, increase in the cost of treatment for frequent hospital visits and dressing material, etc4. Various skin substitutes are autografts, maternal allografts, xenografts, or synthetic tissue-engineered skin. Human Amniotic membrane (hAM), Mesentery, omentum, pericardium, peritoneum, and pleura has been used as natural substitute in a few patients with few challenges in each substitute 5. Commercially available skin substitutes are costly and their availability in developing countries is a big issue. The hAM possesses anti-inflammatory, anti-bacterial, anti-viral, anti-angiogenic, and proapoptotic with various immunological characteristics making it most suitable for allotransplantation6. The hAM is a natural, cheap, easily available skin substitute 7,8,9. We reviewed the literature regarding the use of hAM in paediatric wounds to analyse its feasibility, availability, and safety.

Methods: The literature search was done according to the Preferred Reporting Items for Systematic Reviews (PRISMA) and Meta-Analyses Protocols for the systematic review.

Literature search: We searched the three databases PubMed', 'Web of Science' and 'SCOPUS' with the keywords, "amnion", "biomaterials", "biological dressing", "clinical study", "human amniotic membrane", "paediatric wound", "neonatal wounds", "regenerative medicine". The literature search was extended by snowballing the reference list of all included studies. The period of the search was up to April 2022.

Type of studies: All types of studies animal experimental models, Randomized control trials, Prospective observational studies, case series, and case reports were included.

Inclusion criteria: All studies including patients from the Antenatal period to < 14 years reporting the results of amniotic membrane (AM) biomaterial usage in antenatal/neonatal/paediatric congenital wounds, anatomical defects, and diseases for each patient or the whole group were included.

Exclusion criteria: All studies that included patient data outside the specified age group were excluded. Studies that were reported in languages that could not be adequately translated using Google Translator into the English language were also excluded. Also, the studies in which tissue-engineered skin substitutes were used were excluded

Primary and Secondary outcomes

• Primary outcome was to analyse the results of hAM transplants in different types of paediatric diseases.

• Secondary outcome was to determine the results of wound/defects healing via the usage of "fresh" or "cryopreserved" hAM.

Literature selection and data extraction: The first and second authors independently collected the search results as per Protocol. The duplicated articles were removed in the first step, the abstract of all included articles was read in the second step and data on age, type of wound, type of substitute "fresh" or "cryopreserved" hAM, and effect of wound outcome were extracted in the third step. A common consensus was made on disputed interpretations. Each step was checked by a third author for consistency. The assessment of the third author was considered decisive if consensus could not be reached in the assessment by the first two authors.

Results: One experimental Randomized control trial, three review articles, and twelve case reports (figure 1) were included for final analysis. There was one clinical trial. The experimental studies were in rat, pups and porcine models.

a. Age: The studies of paediatric thermal burn and ophthalmic diseases aged (< 1 month of age) are rarely described in the literature. The youngest child was of 9 months of age, in whom skin graft was harvested from the scalp for the thermal burn 10. The expanded skin grafts have been used in children (6 m-6 ½ yrs) with the youngest child at 6 months of age 11. Two cases of Meningomyelocele (MMC) were repaired with help of autologous

hAM within 24 hours of birth to 3 yrs of age12,13. A case of in-utero repair of MMC is also described in a lamb model14.

a. **Characteristics of hAM:** Following characteristics of hAM make it a suitable material to promote wound healing in large wounds or nonhealing wounds-

1. The hAM functions as a physical barrier against bacterial infiltration by adhesion to the wound surface, thereby reducing the bacterial load[15].
2. The hAM might decrease the risk of infection because it acts as a biological barrier with anti-microbial and anti-viral properties. Cystatin-E an analogue of cysteine proteinase inhibitor has known Antiviral properties[15].
3. The hemostatic property of collagen fibres in the amniotic basement membrane prevents hematoma formation, reducing microbial accumulation and the risk of infection[16].
4. Adhesion of hAM to the wound surface prevents dead space formation, thus preventing discharge accumulation, a nidus of superadded infection [17].
5. Amniotic membrane is nonimmunogenic having anti-inflammatory properties ideal for allografting[17,18,19].
6. The hAM act as a good substrate for epithelial cell growth thus, helping heal the wound.
7. HAM synthesizes numerous growth factors such as epithelial growth factor (EGF), keratinocyte growth factor (KGF), human growth factor (HGF), basic fibroblast growth factor (bFGF), and tissue growth factors (TGF-α, TGF-β-1, TGF-β-2, and TGF-β-3). It also accelerates re-epithelialization and wound healing by the activation of keratinocytes. Healing occurs by re-epithelialization and by complete regeneration of the basement membrane, which plays a decisive role in the integrity and functionality of the skin. The basement membrane is mainly composed of collagen type IV and laminin and is pivotal for coherence between the epithelial and dermal layers [20].
8. The HAM has angiogenesis-promoting factors, which are required for chronic nonhealing wounds [19].
9. The formation of fibrin filaments during wound healing results in the adhesion of the wound to AM collagens, leading to bacterial entrapment and stimulation of phagocyte migration [21].
10. The hAM decreases bacterial proliferation, even in contaminated wounds. Cells of hAM can produce β-defensins, especially β3-defensin, a group of antimicrobial peptides that are expressed on mucosal surfaces by epithelial cells and leucocytes and that belong to the innate immune system Secretory leukocyte proteinase inhibitor (SLPI) and elafin are two elastase inhibitors that are expressed by AM and that possess anti-inflammatory and anti-microbial properties[5,22].
11. Strengthening of hAM by pre-treatment with IL-1 receptor antagonists or lactoferrin results in anti-inflammatory and anti-microbial effects[16].
12. The smooth covering provided by hAM can decrease the loss of protein-rich exudative fluid and loss of excess clear body water by evaporation[17].
13. The hAM is semipermeable, allowing oxygen and water permeation[17].
14. The hAM is transparent, allowing wound surveillance[17].
15. It is nonadherent, so dressing changes are less painful[8,17].
16. The patients were more comfortable with the dressing of HAM as there is less pruritus, need for fewer dressing changes and dressings changes are less painful in children [23].
17. There are fewer requirements for dressing changes. The epithelization of the wound happens beneath the hAM. Once the wound surface is completely re-epithelized the membrane is shed off as eschar[24].
18. The colour of hAM grafted wounds is more hyperpigmented compared to active skin. A study showed paediatric facial burns treated with hAM had a higher incidence of dyspigmentation relative to adult patients (46.2% versus 9.1%, $P \leq 0.05$)[25].
19. No wound contractions have been observable either experimental or clinically in a study on donor site split-thickness skin graft, using dressing material as hAM [19].
20. Few studies quoted scarless wound resurfacing, less possibility of future keloid formation by use of HAM[26].
21. After birth, all perinatal tissues are considered biological waste, which in turn makes them easier to use for other

patients from an ethical perspective[27]. Since HAM is non-tumorigenic, there are no ethical issues for clinical usage [21].

C. Preparation of hAM:

The hAM grafts can be prepared from the human placenta. The mothers from whom the placenta and hAM are to be harvested must be screened for all communicable diseases, viz human immune deficiency virus (HIV), hepatitis C, Hepatitis B, Cytomegalovirus, COVID-19, Treponema Pallidum, and Syphilis. Further the testing of harvested hAM for infectious agents (e.g., agglutination tests for HIV (anti-HIV), hepatitis C virus (anti-HCV), Hepatitis B virus (anti-HBc, HB-Ag), Toxoplasma (anti-), Cytomegalovirus (anti-CMV-IgG/-IgM), and Treponema pallidum hemagglutination antigen testing TPHA- and ELISA-tests for syphilis and polymerase chain reaction tests for HIV, HCV, and HPV and COVID-19. Testing for HTLV-1 and -2 antibodies are usually not needed in an hAM transplant.

Processing of hAM is done by cleaning the placenta with a balanced salt solution containing antibiotics (50 mg/ml penicillin, 50 microgram/ml streptomycin, 100 mg/ml of neomycin, and 2,5 mg/ml of amphotericin B). The membranes are separated and put on a sterile nitrocellulose membrane with an epithelial side of hAM upside. So, when applied to the wound chorionic side would face away from the wound bed. The hydrophobic action of nitrocellulose with protein in the membrane causes immobilization of the membrane.

The hAM is cryopreserved at -80^0 in Dulbecco-modified Eagle media with glycerol (1:1). The AM stored in 50-85% glycerol can be preserved for up to 1 yrs, at 4 degrees Celsius, retaining all native biological properties[928]. To avoid false-negative results, all patients receiving hAM must undergo repeat testing for the above transmissible disease 6 months later[28]. If the placenta is delivered with a history of premature rupture of membrane, meconium staining or the placenta is smelling, it must not be used [29]. The culture of glycerol stored hAM should be done 2 weekly, and if found positive the hAM should not be used for transplantation[29]. Alternatively, hAM can be kept in a sterile bottle with an antibiotic (Gentamycin and Crystalline Penicillin in Normal Saline) and antifungal agents for one Week at 4°C[24]. Some authors used to preserve the hAM in embryo-preserving solutions used during infertility treatment.

The freeze-dried hAM with EDTA in a vacuum pack with sterilization by Gama radiation also retains most of its biological properties[30].

The lyophilized hAM has added the advantage of physical barriers for microbes (Escherichia Coli, Pseudomonas, Citrobacter, Flavimonas, and Staphylococcus)[31].

Lyophilized freeze-dried HAM (Amnioderm) and dehydrated HAM (EpiFix) have been in clinical use as allograft material with standard-of-care protocols for therapy of chronic wounds (wounds > 2 months duration) in adults[2,7,6]. Amnioderm preserves the healing properties of hAM for at least 5 years (shelf-life) at room temperature. Amnioderm is easily available in many developing countries[27]. But the cost is a big issue for people from low socioeconomic status. Hence, comes the role of "fresh" 4^0 C glycerol-preserved hAM.

The advanced treatment of hAM by adding an electrospun layer, or electrospinning or coating of additional layers of hAM, extract of hAM (hydrogels) has been used to enhance the effectiveness and shelf life of AM[32–35]. But the developing world is still struggling with the limited available resources.

For "fresh" hAM collected from the placenta of Caesarean deliveries with due care to maintain proper aseptic conditions during transport and preservation at + 4° C [19]. The " fresh" hAM is equally effective as cryopreserved hAM except they needed maternal testing for communicable diseases at the time of harvesting and retesting at 6 months for transmissible disease. The drawback of "fresh" hAM is the time interval from harvesting hAM to allografting is short and the wastage of unused tissue[36,9]. The 'fresh" hAM is used mostly in ophthalmic (corneal surgeries). Its use in paediatric wounds has not been described so far.

Compared to caesarean deliveries, the hAM derived from the placenta of vaginal delivery (physiological delivery) is rich in EGF and TGF -β. So, fibroblast and keratinocytes rich cell lines are more in the physiologically delivered placenta. The former hAM is more useful in chronic wound healing. On the other hand, the cervical area of HAM retrieved by caesarean delivery has a low level of TGF-β and is more suitable for ophthalmologic applications[16].

D. The procedure of allografting hAM:

Appropriate wound debridement and wound management should be done as per the surgeon's decision. The "fresh" hAM should be applied from the recently delivered placenta (1-2 hours) or 85% glycerol stored hAM at +4^0 C. Before application it must be washed with normal saline. The dull-looking surface of AM (Chorion side) is

kept opposite to the wound surface as chorion might have antigenic properties. No topical antibiotics are recommended[29]. The sofa-tulle dressing can be applied over it, with absorbent dressing and multilayer compression bandage. The cases of extensive thermal burn can be left open, with air-blow dry in of hAM and maintenance of sterile atmosphere around the patient[29].

The hAM can be applied one to two times per week [37]. In case of partial loss of hAM from wound surface area, the lost area can be retransplanted with hAM[29]. In a few cases, an epithelial island appears in the wound bed which enhances wound recovery.

e. Frequency of dressing change in wounds: Frequent dressing changes in wounds with excessive exudate, active infection (wound culture is positive for more than 1 microbes or culture is positive for dermal non-commensal microbes). The frequency of dressing changes can be decreased if granulation and epithelization start appearing on the wound bed. Other factors affecting dressing changes were patient compliance[6].

f. The duration of hAM application in wounds: AM dressings can be changed till the red granulation tissue is replaced by a pale epithelial layer.

g. Type of wounds/ defects: Most of the studies were on paediatric second and third-degree paediatric thermal burn[11,26,25,38]. Six studies on congenital birth defects (meningomyelocele) were identified in which, one study showed antenatal in-utero repair and four were of postnatal repair[14,39,13]. The autologous hAM is the best allograft for dura repair in MMC[13]. The ocular paediatric diseases viz corneal epithelial ulcers, conjunctiva reconstruction following Steven Johnson Syndrome and scarring after surgery of strabismus used AM transplantation[40]. One case report of congenital cutis aplasia[41].

A study on neonates used different biological membranes. The author used hAM at two centres and treated 3 cases of Oesophageal atresia with tracheoesophageal fistula, four cases of congenital diaphragmatic Hernia, two cases of female cloacal anomalies and one case of postoperative fistula formation in a case of Currarino syndrome[42].

h. Outcome of wounds in different paediatric diseases:

In paediatric second and third-degree thermal burns it has been shown to decrease pain, increase the rate of healing, and increase the results of successful skin grafts [26,22]. The combination of the human placental extract with autologous bone marrow MSC achieved better results in wound healing than the placental extract applied without cells [43,44].

In Ophthalmic hAM can be used as a graft on corneal ulcers to induce stromal proliferation and corneal surface epithelization after excision of symblepharon, massive pannus, corneal scarring after strabismus surgery etc [45,40]. In paediatric patients, hAM is used as a patch over inflamed ocular surface viz acute phase of chemical /thermal burn or Steven Jones Syndrome[46]. In deep corneal or scleral ulcers, dual use of hAM is used as a stuffing into small pieces in stromal defects over which AM is used as a graft. All these modes of AM have excellent results in paediatric ocular diseases[30,45].

The autologous cryopreserved hAM was used successfully in human and animal models of meningomyelocele and lipomeningomyelocele. The postnatal surgery using AM side down covering the neural placode prevents the adhesion/ re-teetering of neural tissue with the dura. Further, hAM used extradural facing amnion towards the dura to prevent scaring and thus avoids adhesion between repaired dura and extradural tissue 39,14. Few utero repairs if MMC using hAM have also been reported with insignificant results[14,12].

The hAM was used in Oesophageal atresia with tracheoesophageal fistula as a layer around oesophagal-oesophageal anastomosis to create a separation between the oesophagus and trachea to prevent the risk of recurrent fistula. The hAM also contained the secretion in mediastinum thus decreasing the severity of mediastinitis[42].

The surgical aims were to guarantee a lasting separation between the oesophagus and the trachea in case of dehiscence (three cases) or to cover the oesophageal anastomosis under tension during primary repair (four cases). In CDH patients the human pericardium was used in two cases and fascia lata in three patients. In four patients the membranes were chosen to reinforce the neo-diaphragm and in one patient the fascia lata and PTFE patch were used to close the defect. In ARM patients, the amniotic membrane was used in two female patients with cloaca after the separation of the urinary and genital tract to consolidate the interface between the neo-vagina and urethra. Three out of four patients with Spinal defects had myelomeningocele, which was repaired surgically at birth using the amniotic membrane fixed to the surrounding muscle. In one case, a second amniotic membrane was used to reinforce the first one. The fourth patient was diagnosed with Currarino's syndrome and underwent the procedure of untethering the tethered cord. It healed with a

postoperative fistula that was repaired with a patch of amniotic membrane.

An Experimental Model of Gastroschisis demonstrated Amniotic Membrane uses as a peritoneal substitute having antiadhesive properties. The Amniotic Membrane was put above the bowel surface, between the bowel and skin cover. The AM has been demonstrated to decrease adhesion in 33% of cases. [47]

i.. **Disadvantages of hAM:** It is difficult to obtain hAM at all medical centres. Especial training is required in the procurement and preparation of hAM. The Freeze-dried hAM can be stored at a dedicated cryopreservation centre (deep freezer -80 degrees)[48]. The "fresh" living amniotic membrane, however, presents its challenges, namely, the risk of disease transmission of communicable diseases and the short shelf-life of live amnion[37]. To decrease the chances of transmission of infection "fresh" hAM can be sterilized in an oven[21].

j. Cost Analysis: Cunha et al calculated the cost of time consumed in preparing fresh AM with the calculation of antibiotics, manpower and infrastructure needed to preserve hAM at 4 degrees Celsius for one week. The calculated cost of one placenta was 300 Indian rupees[24].

Discussion: Birth-associated tissues such as the human amniotic membrane (HAM), chorionic membrane, decidua, umbilical cord, and amniotic fluid are traditionally in clinical use for chronic wounds [4944]. The *human umbilical cord mesenchymal stromal cells* (hUC-MSC) can be infiltrated subdermally or subcutaneously. Alternatively, they can be applied topically with collagen scaffolds[49]. The disadvantages of scalp grafts are donor site bleeding, donor wound infection, donor wound contraction, pain, wound morbidity, sparse hairs, and alopecia[1011].

The hAM is usually the waste product of delivery. In 1913 sabella used hAM in the treatment of paediatric thermal burn. During the development of the fetus, the inner layer of the blastocyst provides the hypoblast and epiblast, the latter producing the amniotic epithelial layer. Since the epiblast is also the source of all of the germ layers of the embryo, the layer of amniotic epithelial cells also possesses a wide differentiation. Fetal membranes are composed of two layers: an outer layer (chorion), which contacts maternal cells, and an inner layer (amniotic membrane; AM). AM or amnion is a thin membrane on the inner side of the placenta; it surrounds the fetus and delimits the amniotic cavity, which is filled with amniotic fluid. The hAM possesses anti-inflammatory, anti-bacterial, anti-viral, immunological characteristics, and anti-angiogenic and proapoptotic features. Hence it is ideal for allotransplantation[2149].

Human amniotic membrane grafting is used as an adjunctive by ophthalmologists, dentists, urologists, burn specialists, otorhinologist, gynaecologists, and researchers in stem cell technology[18]. The antiadhesive and antimicrobial properties of hAM could be utilized in paediatric surgical diseases that needed extensive pelvic dissection for Altman type 3 type 4 Sacrococcygeal teratoma, redo cases of Anorectal malformation. recurrent rectourinary fistula [50], vesicovaginal fistula [50], redo surgeries for Hirschsprung disease, vaginal reconstruction etc.

Difficult wounds viz peristomal excoriation [51] and resurfacing of oral mucosa could be performed after buccal/ lingual mucosa harvest in Bracka's staged repair in hypospadias cripple.

An experimental study demonstrated an increased strengthening of colonic anastomosis and fewer postoperative adhesions in LASER-treated AM-wrapped colonic anastomosis[52]. Contrarily, an experimental study showed no advantage of hAM in postoperative adhesion formation following multilayer AM application to colonic defects[53].

A systematic review based on three experimental models described safe and effective anatomical, functional results with less postoperative adhesions in cases of ischemic bowel disease and Colitis in whom the mesenchymal stem cell has been used as local injections, coating of suture material by stem cell or systematic injection[54,55]. These results can be expanded over the neonates who underwent primary ischemic intestinal anastomosis viz cases of multisegmental Necrotizing enterocolitis.

The fetus swallows the amniotic fluid, thus the intestine gets exposed to many trophic factors of amniotic fluid. But, the intestines of preterm babies are deprived of trophic factors. The supplementation of formula feeds with 30% amniotic fluid extracts provides amniotic fluid-borne growth factors, and cytokines and prevents experimental NEC in rat pups[57]. The author suggests oral supplementation of amniotic fluid in neonates having uncomplicated or complicated NEC. The autologous hAM of prematurely delivered babies can be preserved, for possible utilisation in cases of complicated NEC needing surgical interventions. Further research in this field is suggested as it can have the potential for a better outcome [58,54].

Necrotizing fasciitis (NF) is a potentially life-threatening infection characterized by the rapid spread of inflammation and infection with widespread necrosis of fascia, subcutaneous

tissues, and overlying skin. Contrarily to adults, neonates having NF are usually healthy. It is proposed that routine care of neonates causes minor breath in the continuity of the epidermis, which gets infected and predisposes to NF [3]. The first author successfully used fresh hAM in one postoperative case of gastroschisis, who developed partial flap necrosis of raised abdominal flaps (fig 1).

The hAM can be used in many forms fresh, intact, dried, frozen, and extracted (decellularized). In-Vitro studies proved that fresh hAM is more suitable for cellular therapy and clinical usage compared to cryopreserved hAM because fresh hAM retains all its function (regenerative potential and tensile strength) in the long term[48]. It can be used in acute or chronic paediatric wounds [5]. The hAM can be used directly over wounds or it can be used to grow stem cells by different culture methods [5,37].

The advantages of hAM are its easy availability in large quantities at hospitals having maternal and child care facilities[17]. The hospital stay, infection rate, antibiotic duration, cost of dressing material or operation for split-thickness skin grafting, and anaesthesia risk are less in patients treated by use of hAM in either acute or chronic wounds, the hAM can be safely used in paediatric wounds[29,24]. The stem cell differentiation and antiadhesive properties of hAM are used in congenital birth defects having the risk of adhesive intestinal obstruction[42].

Allogeneic AMs are not extensively used in many European countries because of regulatory restrictions on human tissue products and cost considerations favouring alternative products[59]. Aseptic refers to using methods to prevent, restrict, or minimize contamination with microorganisms from the environment, processing personnel, or equipment without sterility assurance, whereas sterile refers to a terminal sterilization process validated to a sterility assurance level of 10−6 [60,61]. This has led to a debate on infections post-surgical implantations in AMs. To overcome the issues of sterility, some countries introduced electron-beam sterilization as a sterile and non-immunogenic AM, which eliminates viruses, bacteria, and spores, achieving sterility levels.

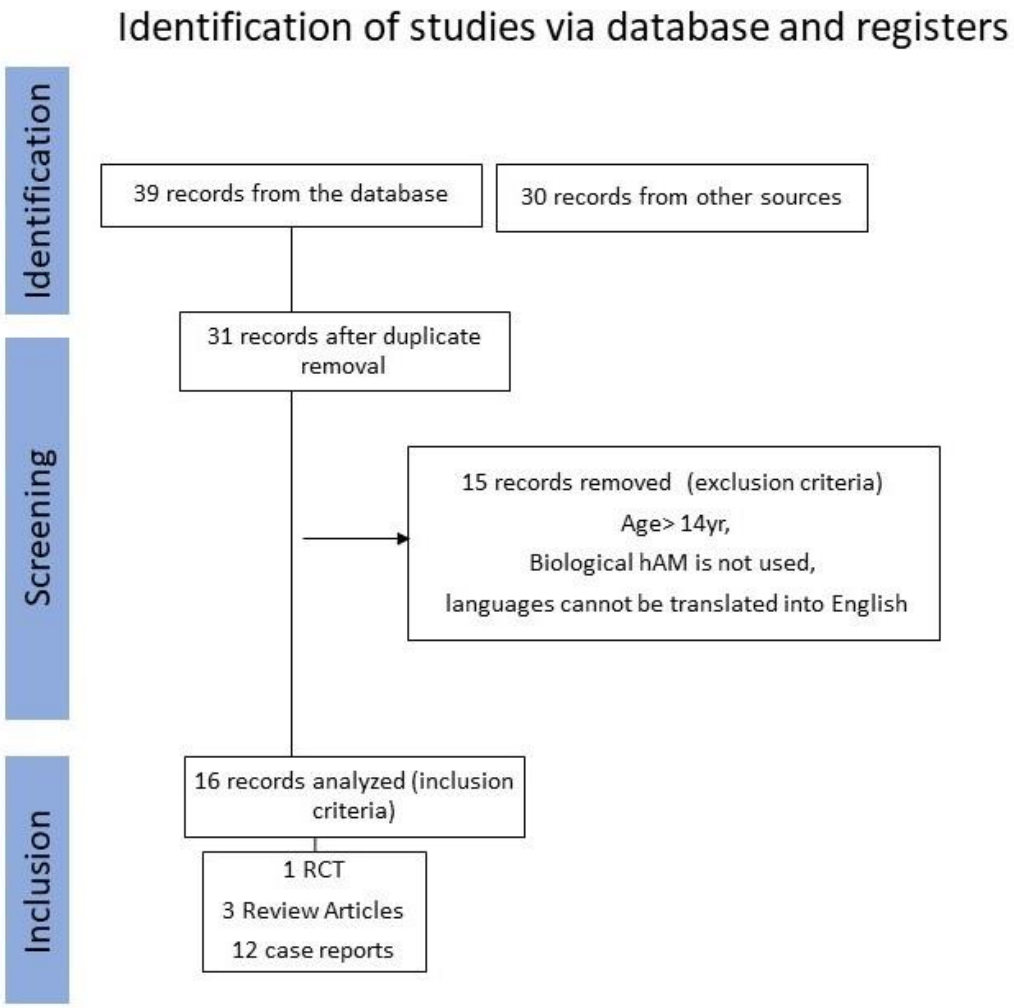
The World Health Organization's regulations, on Key Safety Requirements for Essential Minimally Processed Human Cells and Tissues for Transplantation and Resolution – Human Organ and Tissue Transplantation, are based on the principle of non-commercialization of human cells, tissues and organs for transplantation[62]. In addition, it also addresses ethical issues relating to tissue recovery for transplantation, procedures to be followed to uphold the quality and safety of tissues, and encourages collaboration in information sharing, especially with regard to adverse events and post-transplantation adverse reactions[61].

There is noticeable heterogenicity in the literature available on paediatric surgical diseases. There is a need to standardize the methods of isolation of hAM in clinical usage resource-limited studies. Further, experimental and clinical studies are needed to expand the indications, administration route, and potential adjuvants to establish the safety and efficacy of hAM usage in paediatric surgical diseases.

Abbreviations:

bFGF: Basic fibroblast growth factor
COVID-19: Sars Novel Corona Virus -19
EGF: epithelial growth factor
HGF: human growth factor
HIV: Human immunodeficiency virus
HCV: Hepatitic C Virus
HPV: Human Papilloma Virus
hAM: Human Amniotic membrane
hUC- MSC: The *human umbilical cord mesenchymal stromal cells*
KGF: keratinocyte growth factor
LASER:
MMC: Meningomyelocele
NEC: Necrotizing enrterocolitis
TGF: tissue growth factors (TGF-α, TGF-β-1, TGF-β-2, and TGF-β-3)

Figure 1: PRISMA Flow diagram (PRISMA: Preferred Reporting Item in Systematic Review and Meta-analysis).



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