Randomized trial to evaluate and compare the immunogenicity and safety of hexavalent vaccine in healthy infants in a polio-endemic country.

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1. Background

The development and widespread use of vaccines to prevent infant diseases have been one of the most significant public health advancements in recent history. Immunization has saved millions of lives and prevented countless more from suffering from serious health complications. However, as more vaccines are introduced into immunization programs worldwide, it has become increasingly challenging for healthcare systems and parents to manage the process effectively.

To address this issue, the use of combination vaccines has emerged as an effective solution. These vaccines combine multiple antigens into a single shot, which has several advantages over administering individual vaccines. Firstly, it reduces the economic burden on parents and healthcare systems, as they only have to pay for and administer one injection instead of multiple.(1) Additionally, combination vaccines can improve compliance with vaccination schedules since they reduce the number of visits needed to complete the full vaccination schedule.

The use of combination vaccines has been shown to have a positive impact on public health, especially in developing countries where healthcare resources are limited. By reducing the number of injections needed, combination vaccines have made it easier for parents to ensure their children are vaccinated on time, which has led to a reduction in infant mortality rates. Overall, combination vaccines have played a significant role in improving the efficacy and accessibility of immunization programs globally.

Polio is a viral disease caused by the poliovirus, which mainly affects young children. Pakistan is one of the last two remaining countries in the world where polio is still prevalent (2). It is a matter of great concern as in 2023, a total of six cases of Wild Polio Virus 1 were reported in the country, along with 98 positive environmental surveillance. Therefore, it is extremely crucial to include both oral polio vaccine (OPV) and inactivated polio vaccine (IPV) in the immunization schedule to combat the spread of the disease (3).

The current extended program for immunization schedule in Pakistan includes OPV at birth, 6 weeks, and 10 weeks, with IPV administration at 14 weeks and 9 months. The addition of the IPV vaccination has significantly increased the number of injections required for immunization. However, it is important to note that the benefits of the IPV vaccine outweigh the inconvenience of additional injections. It provides additional protection against the disease and helps in reducing the spread of polio. Therefore, it is recommended to follow the immunization schedule strictly to protect children from the disease.

The introduction of the hexavalent vaccine is a significant advancement in global public health efforts (4). This vaccine offers a comprehensive solution to prevent six major infectious diseases that affect children. These diseases are diphtheria, tetanus, pertussis (whooping cough), hepatitis B, Haemophilus influenzae

type b (Hib), and polio. The consolidated vaccine is expected to increase compliance among patients as it reduces the number of injection encounters. By consolidating the vaccine, healthcare resources such as personnel, transportation, and storage can be optimized, making it more sustainable, especially in resource-limited settings.

It is believed that the current use of acellular pertussis in vaccines may result in a weaker response compared to vaccines that use whole-cell pertussis (5). However, there are currently no whole-cell pertussis vaccines available for the hexavalent vaccine that can be used as routine immunization.

The primary objective of this study is to evaluate the immunogenicity of the newly prequalified Hexavalent vaccine in comparison to the current EPI pentavalent vaccine in routine vaccination. The focus is to determine whether the new vaccine is non-inferior to the current vaccine. The study design includes randomized controlled trials which will be conducted on a large population of individuals across multiple sites. The study will use rigorous statistical methods to analyze the data collected from the trials. The study will also assess the safety of the new vaccine in comparison to the current vaccine. The results of this study will provide valuable insights into the effectiveness of the new vaccine and its potential to replace the current vaccine, thereby improving global vaccination efforts.

2. **Rationale:**

It is believed that the current use of acellular pertussis in vaccines may result in a weaker response compared to vaccines that use whole-cell pertussis (5). However, there are currently no whole-cell pertussis vaccines available for the hexavalent vaccine that can be used as routine immunization. The primary objective of this study is to evaluate the immunogenicity of the newly prequalified Hexavalent vaccine in comparison to the current EPI pentavalent vaccine in routine vaccination (6). The results of this study will provide valuable insights into the effectiveness of the new vaccine and its potential to replace the current vaccine, thereby improving global vaccination efforts.

3. **Study Objectives**

Primary

a. To compare the humoral immunity against type 1-3 poliovirus after administering 3 doses of the Hexavalent schedule (Arm A) and compare it with the national EPI schedule of pentavalent + IPV administered at 6, 10, and 14 weeks of age (Arm B).
b. To compare the humoral immunity against type 1-3 poliovirus after administering 3 doses of the Hexavalent schedule (Arm C) and compare it with routine immunization pentavalent + IPV administered at 2, 4, and 6 months of age (Arm D).

c. To compare the humoral immunity against type 1-3 poliovirus after administering 4 doses of the Hexavalent schedule given according to the national EPI schedule at 6, 10, 14 weeks, and 9 months of age (Group I) with Hexavalent doses given at 2, 4, 6, and 12 months of age (Group II).

Secondary

a. To evaluate the humoral immunity against type 1-3 poliovirus after administering the Hexavalent vaccine schedule at 6, 10, and 14 weeks (Arm A) and 2, 4, and 6-month schedule (Arm C).

4. **Methodology**

Study Design

The study will be an open labelled randomized vaccine trial in Pakistan comparing the new hexavalent Hexasil (SII) vaccine to currently used vaccines i.e. pentavalent + IPV in routine immunization in Expanded Program of Immunization in healthy infants between 6-8 weeks old at the time of enrolment.

Study Population and Sites

The Clinical Trial Unit (CTU) of Aga Khan University Hospital in Karachi, Sindh, Pakistan will serve as the designated location for conducting the study. Infant participants will be enrolled either at 6 weeks in Group I or at 2 months 8 in Group II. The study will recruit participants from two peri-urban districts of Karachi, namely Cattle Colony and Ibrahim Hyderi, as well as the Aga Khan Hospital for Women and Children in Kharadar. These areas were selected as they represent a diverse population and will provide a reliable sample for the study (7).

Description of the study population and setting to be studied.

Karachi is a vast city that comprises five districts and eighteen towns. The study we are conducting will take place in two peri-urban and adjacent coastal villages outside Karachi, namely Cattle Colony and Ibrahim Hyderi. These villages are situated at a driving distance of around thirty minutes from AKU, where the Department of Paediatrics and Child Health is established. This department has a well-established demographic surveillance system that captures all pregnancies and births in the area. According to our census in 2023, the population of these communities was approximately 172,815, and the annual birth cohort is approximately 5100. The surveillance system is the basic infrastructure for conducting many internationally funded studies and interventions related to neonatal infections, respiratory, enteric infections, and poliomyelitis vaccine trials. This system is funded through the WHO, Gates Foundation, GAVI Alliance, PATH, NIH, and other organizations. There are around 150 community health workers (CHWs) involved in active surveillance activities. This team captures five events, including the birth of a

newborn, the death of children under five, new pregnancy, and in and out-migration from all households in the surveillance areas. On average, ≈426 newborns and 475 pregnant women are being captured in a month. The team has good communication with traditional birth attendants that helps in early birth notification. Sixty percent of births occur at home, while the remaining births occur in on-site maternity clinics. Each area has a Primary Health Centre (PHC) run by the Department of Paediatrics and Child Health research program. The centre also provides Expanded Programme of Immunization (EPI) services in collaboration with the local town health officer, who provides the EPI vaccines. There are three sites where EPI vaccines are given or at the government-run EPI facility located next door in one of the four proposed sites. Area hospitals/clinics, including Sindh Government Hospital, Koohi Goth Hospital, and Ma Ayesha Trust Centre, also serve as sentinel sites for capturing diseases in the surveillance system.

Screening and Enrolment Process

Infants between the ages of 4-5 weeks will be identified from the peri-urban sites with the help of a Demographic Surveillance System which is being run by the paediatric department of Aga Khan Hospital. They will also be identified from the birth records at the Aga Khan Hospital for Women and Children, Kharadar. The study staff will approach the parents and/or guardians, explain the study in detail and obtain verbal consent. If they agree to participate, they will be considered eligible for the screening and subsequent enrollment.

At the time of enrollment, which will be when the infant is 6 weeks old, the parents will be asked for written consent. The study processes will be explained to them in detail, including the number of visits required, the blood sampling process, and what will be done with the blood samples. Once the parents sign the written consent, the child will be randomized into one of four groups and will be vaccinated accordingly.

Eligibility Criteria

Inclusion Criteria:

- All healthy full-term infants (born at 37 weeks) at 6-8 weeks old of either gender.
- Not planning to travel away during entire the study period (enrolment- appoximately 294 days; 5 weeks – 42 weeks).
- Parents resident of the study area for the last 3 months at the time of enrolment
- Parent/guardian provides informed consent.

Exclusion Criteria:

- Newborns found acutely ill at the time of enrolment and requiring emergent medical care/hospitalization.

- Enrolment weight & height <-3 weight for height z-score.
- Infants with certain medical conditions i.e., syndromic infants, infants with petechial, purpura, or bleeding disorder (contraindication of intramuscular injections)
- A diagnosis or suspicion of immunodeficiency disorder (either in the participant or in a member of the immediate family - e.g. several early infant deaths, a household member on chemotherapy) will render the newborn ineligible for the study.
- Diagnosed neurological disorder or a history of seizures or temperature >38^0C in last 3 days or any other indication of acute illness/infection within the past 7 days.
- Having a record of anaphylaxis or allergy to vaccine components.

5. **Data Collection and Management:**

Tablets will be used for recording data for each participant enrolled in the study. The site investigators will ensure the accuracy, completeness, legibility, and timeliness of the data captured in eCRF. Data captured in the eCRF if derived from source documents and should be consistent with those source documents.

A unique username issued by the clinical data management provider and an associated password will be required to log into the Electronic Data Capture system to access study data. The server will have a backup system. There is a logging system for collecting action with logs recorded in the system. At the user end, we will have a daily, weekly, and monthly backup system to back up all the data in a local computer. The data will be stored on a main server.

Data Confidentiality and Privacy:

Privacy and the confidentiality of the child's data will be protected by storing the data in locked cabinets and password protected computers. It will only be accessible to authorized study staff. If it becomes necessary for the Ethics Review Committee to review the study records, information that can be linked to the child will be protected

Source Documents:

eCRFs will be used for recording data for each participant enrolled in the study. The site investigators are responsible to ensure the accuracy, completeness, legibility, and timeliness of the data captured in eCRF. Data captured in the eCRF derived from source documents and should remain consistent with those source documents. In case of discrepancies, data will be clarified and corrected.

Study staff will extract all data collected in source documents and workbooks for entry into the eCRF. An Electronic Data Capture (EDC) system is a software that will be used to store the participant's data collected in this study

6. **Study Vaccines:**

The hexavalent vaccine Hexasiil manufactured by the Serum Institute of India is a single dose injection (0.5 ml) comprising of the following constituents: Diphtheria Toxoid ≤ 30 IU, Tetanus Toxoid ≥ 40 IU,- B. pertussis (whole cell) ≥ 4 IU, HBsAg (rDNA) 15 mcg, Inactivated polio vaccine (Salk strains grown on vero cells) Type - 1 (Mahoney strain) 40 DU, Type - 2 (MEF-1 strain) 8 DU, Type - 3 (Saukett strain) 32 DU, Hib (PRP) 10 mcg, conjugated to TT (carrier protein) 19 to 33 mcg

The pentavalent vaccine currently being used in the EPI schedule is manufactured by Biological E Limited. Each Dose of 0.5 mL contains: S Diphtheria Toxoid 25 Lf (≥ 30 IU); S Tetanus Toxoid 5.5 Lf (≥ 60 IU) S B. Pertussis (Whole cell) 16 IOU (≥ 4 IU); S r-HBsAg 12.5 µg S Puried Capsular Polysaccharide of Hib (PRP) covalently linked to 20 to 36.7 µg of Tetanus Toxoid 11 µg S AI (as AIPO) 4 ≤ 1.25 mg S Preservative: Thiomersal BP/ Ph.Eur 0.01% w/v

Imovax Polio_ comprised of inactivated Salk Polio Virus (Type 1 = 40 DU, Type 2 = 8 DU, Type 3 = 32 DU); aluminum phosphate gel (_1.25 mg) and thiomersal (0.005%).

7. **Accountability**

The investigator or designee is responsible for study intervention accountability, reconciliation, and record maintenance (i.e., receipt, reconciliation, and final disposition records). All study interventions will be accounted for using a study intervention accountability form/record.

Unused study interventions must be available for verification during on-site monitoring visits. Unused study interventions will be destroyed on-site and documented on the Investigational Product (IP) accountability form.

8. **Handling and processing of study samples:**

Blood

Three (3) ml of peripheral venipuncture blood will be collected by a trained phlebotomist at four point times (Refer to table). Blood samples will be transported to AKU for processing and serum separation. Sera will be stored in AKU - infectious diseases research laboratory (IDRL) AT -80 ^{0}C until transfer to NIH, Pakistan for measurement of neutralizing poliovirus antibody.

The humoral assays will be run at NIH Islamabad where they will be analyzed using standard microneutralization assays against polio antibodies for all three serotypes

9. **Safety Considerations**

Exclusion Criteria for the Subsequent Vaccination

If any of the following occurs (1-3), it's forbidden to continue vaccination, but other study procedures can be continued according to the investigator's judgment.

1) Any newly confirmed or suspected autoimmune disease or immunodeficiency disorders.
2) Any serious adverse events related to vaccination in this study.
3) Severe allergic reactions after vaccination in this study.

If the following event occurs, it is up to the investigator to decide whether to vaccinate or not.

4) Other reactions (e.g., severe pain, severe swelling, severe activity limitation, persistent high fever, etc.) that are considered to influence the vaccination according to the investigator's judgment.

If the following (5-6) occurs, the vaccination could be postponed within the required time window.

5) Various acute diseases or acute exacerbations of chronic diseases by the time of vaccination.
6) Axillary temperature ≥37.3℃

Adverse and Serious Adverse Events (AEs and SAEs)

Adverse Event (AE)

In this study, an AE will be defined as any untoward medical occurrence that occurs in a participant who is vaccinated and that does not necessarily have a causal relationship with the vaccine's administration.

Solicited/Unsolicited adverse events

In this study, the solicited period is from Day 0 to Day 7 after each vaccination. The unsolicited period is from Day 8 to Day 28 after vaccination. Solicited AEs are used to assess the reactogenicity of the study vaccine and are predefined local (at injection site) and systemic events for which the participant is specifically questioned. Unsolicited AEs refer to the unsolicited symptoms occuring within the solicited period, or all symptoms occurring within the non-solicitation period.

Solicited local (injection site) symptoms:

Redness, swelling, rashes, induration, and pruritus

Solicited systemic symptoms:

Fever (axillary temperature will be measured), acute allergic reaction, diarrhea, decreased appetite, irritability, decreased activity

Serious Adverse Events (SAEs)

A SAE is an AE that results in any of the following outcomes, whether or not it is considered to be related to the study intervention.

- Death.

- Life-threatening event (i.e., means that the subject is at risk of death at the time of the onset of the adverse event).
- Hospitalization or prolongation of existing hospitalization, regardless of length of stay, even if it is a precautionary measure for continued observation. Hospitalization for a pre-existing condition that has not worsened unexpectedly does not constitute a serious AE.
- Persistent or significant disability or incapacity resulting in a substantial disruption of a person's ability to conduct normal life functions.
- An important medical event (that may not cause death, be life threatening, or require hospitalization) that may, based upon appropriate medical judgment, jeopardize the volunteer and/or require medical or surgical intervention. Examples of such medical events include allergic reaction requiring intensive treatment in an emergency room or clinic, blood dyscrasias, or convulsions that do not result in inpatient hospitalization.

10. **Screening Failures**

Participants who consent to participate in the clinical study but do not subsequently receive the study intervention will be considered as screening failures. Screening failure details such as why they did not receive the intervention are required to ensure transparency of enrolment procedures.

11. **Randomization and Blinding**

Randomization

The enrolled participants will be randomly separated into four at a 1:1:1:1 ratio. Stratified Blocked Randomization will be carried out, taking the study site as a stratification factor. The independent randomization statistician will use SAS software to generate a randomization code list. For each eligible participant, the grouping information will be revealed after enrolment including the name of the vaccine and the site of vaccination.

Blinding

This study will be an open-labeled design, as blinding will not be possible. However, during blood collection, the Phlebotomist is not aware of the participant group to reduce bias. In addition, lab personnel will be completely blind during the laboratory assessment as each sample will be labeled with a unique ID code without grouping information.

12. **Concomitant Medications and Therapies**

- Necessary drug therapy and medical treatment are to be given in case of any adverse events including serious adverse events.

- A detailed record of any concomitant medicine given during the study, including name, doses, duration, etc will be maintained.
- Vaccines for an emergency medical indications, such as rabies; will be allowed, However, they are prohibited from receiving any OPV (other than birth) and immunization vaccines *other than* EPI scheduled vaccines and the study vaccines. That is no supplementary immunization vaccines are to be administered.
- Administration of any vaccines other than the study vaccine during the study should be recorded in detail, including name, dosage and usage, date, etc.

13. **Safety Oversight**

Safety Assessments

Planned time points for all safety assessments are provided in the Schedule of Activities. Unscheduled clinical laboratory measurements may be obtained at any time during the study to assess in case there are any perceived safety issues. Any clinically relevant changes occurring during the study must be recorded on the AE section of the eCRF.

Safety Follow-up

All participants are required to be observed for 30 minutes on site after the last vaccination in each vaccination visit. Diary cards will be distributed to guardians of participants to record AEs from the time of first vaccination to up to 28 days after vaccination. Measurement, recording, precautions, and reporting methods of AEs will be explained to the participant. Guardians of participants are required to closely observe participants' symptoms and fill the diary every day.

The parents/LARs will be asked to record the AEs at any time. Any acute allergic reactions, AEs with a severity of grade 3 and above, and SAEs should be reported to the investigator as soon as possible. It will be the responsibility of the investigator to follow up by self or a designee throughout and after the active collection period and until the AE or SAE or its sequelae resolve or stabilize.

SAEs will be reported to the DSMB and ERC within 24 hours of information. Regulatory authorities will be informed within 2 working days. Follow-up information should include a description of the event in sufficient detail to allow for a complete independent medical assessment of the case and a determination of possible causality.

Participants Withdrawal from the Study

A participant may withdraw from the study at any time as stipulated in the consent form. The reason for withdrawal will be recorded in the CRF. In case of participant withdrawal, any data and blood samples collected before their withdrawal will still be used in the analysis.

If withdrawal is due to any adverse event resulting from vaccination, appropriate medical care will be provided till resolution unless the participant refuses treatment.

Lost to Follow-up

If a participant repeatedly fails to return for scheduled visits or is unable to be contacted by the study staff, all efforts should be made to contact the parents/LAR and reschedule the missed visit. All attempts must be documented and/or a note made in the file. If there is no contact with the participants after visits, then the child is considered as lost to follow up.

Study Pause and Early Termination

If more than 15% of the participants have grade 3 or severe adverse reactions, including local or systemic reactions, the principal investigator or DSMB may call for a pause in the study. After reporting to the ERC and regulatory bodies, if it is deemed necessary, the study may be terminated with a complete explanation of the reason.

14. **Sample Size:**

The sample size needed to demonstrate that the hexavalent vaccine is non-inferior to the pentavalent + IPV vaccine, with a 10% margin at the EPI schedule, was calculated to be at least 434 (rounded to 440) participants per group. This calculation was based on 90% power, a 5% significance level, and 25% mucosal immunity in both comparison groups.

The details of the sample size are provided in the table below:

Mucosal immunity proportion in pentavalent + IPV arm	0.25	0.25	0.85
Mucosal immunity proportion in hexavalent arm	0.25	0.25	0.85
Observed/Expected difference in proportions	0	0	0
Non-inferiority margin	10%	10%	10%
Power (1- beta) %	90	90	90
Alpha Error %	5	2.5	2.5
Required sample size in each group	321	369	268
15% attrition	378	434	315

Table 1: Study Group

	Baseline	6 weeks	10 weeks	14 weeks	14 weeks	28 days post-vaccination	9 months	28 days post-vaccination
Arm A (n=440)	**Blood**	Hexavalent + OPV	Hexavalent + OPV	**Blood**	Hexavalent + OPV	**Blood**	Hexavalent	**Blood**
Arm B (n=440)	**Blood**	Pentavalent + OPV	Pentavalent + OPV	**Blood**	Pentavalent + OPV + IPV I	**Blood**	IPV II	**Blood**
Study Group II								
	Baseline	**2 months**	**4 months**	**14 weeks**	**6 months**	**28 days post-vaccination**	**9 months**	**28 days post-vaccination**
Arm C (n=440)	**Blood**	Hexavalent + OPV	Hexavalent + OPV	**Blood**	Hexavalent + OPV	**Blood**	Hexavalent	**Blood**
Arm D (n=440)	**Blood**	Pentavalent + OPV	Pentavalent + OPV	**Blood**	Pentavalent + OPV + IPV I	**Blood**	IPV II	**Blood**

15. **Study Record Retention**

The site investigators (SI) will retain all study records that support eCRFs for this study (i.e., ICFs, source documents, investigational product dispensing records) required by sponsor and by the applicable regulations in a secure and safe facility. These documents will be retained for at least 7 years after the study completion and the electronic records for a period of 25 years as per AKUH clinical trial data retention policy.

16. **Quality assurance:**

The trial will be consudcted in compliance with the protocol, good clinical practices (GCP) and all study staff handling study procedures will have GCP certification. Ethical approval has been obtained from Aga Khan University ERC, Drug Regulatory Authority of Pakistan, WHO IRB, and National Bioethics Committee of Pakistan.

An independent DSMB will be constituted which will comprise experts from various fields of medicine who are independent of the study. The DSMB will meet The DSMB will have the authority to halt or terminate the study in case of any safety concerns.

17. **Statistical Analysis Plan:**

Seroprevalence will be reported as percentages with corresponding 95% confidence intervals (CI) for both groups. A permutation test, adjusted for multiple comparisons using the Bonferroni correction, will be used to compare the two proportions. Antibody titers will be presented as medians, accompanied by percentile bootstrap CIs. The Wilcoxon Rank sum test will be applied to compare the median antibody titers between the two arms.

To compare seroprevalence across age groups and between the two arms, as well as median antibody titers, Generalized Estimating Equations (GEE) with an exchangeable correlation matrix will be applied. Additionally, the Log Rank test will be used to compare the distribution of antibody titers between the two arms.

The same analysis methods will be applied to Study Group II.

18. **Dissemination and publication policy**

Results from the study will be disseminated through multiple channels to ensure broad and meaningful impact. In addition to publication in open access peer-reviewed scientific journals and presentation at academic conferences, findings will be shared with the participating communities in an accessible and culturally appropriate format. Results will also be communicated to local and national polio program as well as policy makers to support evidence-informed decision-making regarding immunization programs.

A clear publication policy will be followed for all study outputs. The Principal Investigator will lead the preparation and submission of manuscripts, in consultation with the study team. Authorship will be assigned according to established ICMJE criteria, and any contributors who do not meet authorship criteria will be acknowledged appropriately. The sequence of authorship

and responsibilities for drafting, reviewing, and approving manuscripts will be agreed upon by all key collaborators prior to submission.

19.**Anticipated Problems**

An anticipated challenge is the potential for enrolled children to immunizations outside the study schedule, particularly during Supplementary Immunization Activities (SIAs), such as door-to-door polio campaigns or other routine outreach efforts. Such unscheduled vaccinations could affect the integrity of the study data and compromise the accuracy of immunogenicity and safety assessments.

To minimize this risk, the study team will proactively communicate with the parents and guardians who will be thoroughly informed about the importance of adhering strictly to the study's vaccination schedule and avoiding any non-study immunizations during the trial period. Regular reminders and clear written instructions will be provided at each visit.

Additonally, close coordination will be maintained with local health authorities, including the Expanded Programme on Immunization (EPI) and the Polio Eradication Program, to reduce the likelihood of inadvertent vaccination during SIAs. Where feasible, local vaccinators will be informed about the study and advised to confirm participation status before administering vaccines.

20.**Benefit to the GPEI**

The hexavalent vaccine is a significant advancement in global public health efforts. The consolidated vaccine is expected to increase compliance among patients and optimize healthcare resources, especially in resource-limited settings. The results of this study will provide valuable insights into the effectiveness of the new hexavalent vaccine and its potential to replace the current pentavalent vaccine, thereby aiding global vaccination efforts and improving coverage.

21. **Study Timeline:**

Table 2.

Study Timeline

Actions	Timeline

AKU ERC, WHO ERC, and National Bioethics Committee approvals. staff recruitment, training of staff, development of a manual of operations and other study materials, procurement of vaccines	**2 months**				
Pretesting, Identification, and recruitment of eligible, intervention, Field activities		**8 months**			
Study participant's Follow-up visits			**10 months after the last recruitment**		
Laboratory analysis				**3 months**	
Data analysis and report writing					**1 month**
					Study duration 24 months

22. References

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