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ORIGINAL ARTICLE

Evaluation of Vaccines Injection Order on Pain Score of Intramuscular Injection of Diphtheria, Whole Cell Pertussis and Tetanus Vaccine

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Abstract
Objective To determine, whether or not intramuscular injection of diphtheria, pertussis and tetanus (DwPT) vaccine should be given first and subcutaneous injection of measles, mumps and rubella (MMR) thereafter or vice versa and can this cause less pain of DwPT vaccine injection.
Methods In a randomized parallel group clinical trial, seventy 18-mo-old healthy children who were referred for routine vaccination to Akbari Health Care Center, Yazd, Iran from September 2014 through March 2015 were randomly allocated to two groups to receive DwPT and then MMR vaccines or MMR first, and then DwPT. Primary outcomes included pain score during DwPT injection, pain score during MMR injection, overall pain score of vaccination and obtaining a pain score of less than three during DwPT injection. Pain scores were assessed based on Modified Behavioral Pain Scale. Secondary outcome was crying duration during DwPT injection.
Results Thirty seven girls and 33 boys were evaluated in two groups. Pain scores of DwPT and MMR injections, the frequency of pain score obtained to be less than three during DwPT injection and the crying duration were not different in both groups. But, overall pain score of vaccination was lower when subcutaneous injection of MMR vaccine was given before intramuscular injection of DwPT vaccine. (14.23 ± 1.35 *vs.* 15.61 ± 2.65; $P = 0.04$).
Conclusions Overall pain score of vaccination in multiple vaccine injection at the same visit might be reduced if subcutaneous vaccine is injected before intramuscular one.

Keywords Vaccination · Pain · Child · Order · DwPT · MMR

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Introduction

Routine vaccine injections that may be administered intramuscularly, subcutaneously or intradermally are the most common sources of iatrogenic pain during childhood [1] and accompanied fear and distress with vaccine injections are among the most important reasons of parents' reluctance to perform timely vaccinations [2]. Untreated pain can negatively affect the development of the central nervous system and has long standing consequences, including procedural anxiety, hyperalgesia, fear of needle and avoidance of health care [3]. Therefore, control, management and decrease of immunization pain and its emotional and physical effects on children and decrease of parental anxiety, during and soon after vaccination by health professionals, are necessary [4]. Many pharmacological and non-pharmacologic interventions or combination of pharmacological and physical and psychological methods have been recommended to reduce immunization pain in children [1, 5].

Intramuscular (IM) vaccine injection is the most painful one and some studies have shown that longer needles are usually accompanied with less pain and less local reaction [1]. Ipp et al. in their research concluded that pragmatic rapid injection technique (no aspiration, rapid injection and rapid withdrawal) is less painful than standard technique (slow advancement of needle into the muscle, slow aspiration prior to injection, slow injection time and slow withdrawal of needle after injection) in IM injection of

DPTaP-Hib (diphtheria, polio, tetanus, acellular pertussis and Haemophilus influenzae) vaccines [6]. Rapid IM injection of vaccine without aspiration can probably cut the pain by combined effects of reducing the time of contact between needle and tissue and curbing within the tissue the lateral movement of the needle [3].

Today, the number of recommended necessary vaccines have increased and sometimes infants or children should receive two or more vaccine injections during the same visit [1, 3].

According to vaccination programs of the Ministry of Health and Medical Education of I.R. Iran, all 18-mo-old children should routinely receive intramuscular injection of diphtheria, whole cell pertussis and tetanus (DwPT) as well as subcutaneous injection of measles, mumps and rubella (MMR) in primary health care centers, at the same time.

Some researchers suggest to inject the most painful vaccine last, to minimize priming the effect of the first injection on subsequent injections, and thus, reducing the overall pain when multiple vaccine injections (intramuscularly and subcutaneously) should be administered to the children at the same visit. Optimal order of injection for vaccines which are administrated at the same visit, however, has little evidence [3].

The present study was done to answer the question whether or not intramuscular injection of DwPT vaccine should be administered first and subcutaneous injection of MMR vaccine hereafter or *vice versa*, and can this cause less pain of DwPT vaccine injection. The aim of the present study was to evaluate the effect of the order of injection (DwPT and MMR or MMR and DwPT) on pain of intramuscular DwPT vaccine at 18 mo of age.

Material and Methods

In a randomized not-blinded parallel group clinical trial, all consecutive 18-mo-old healthy children who were referred for vaccination to Primary Health Care Center of Akbari, Yazd, Iran from September 2014 through March 2015, were enrolled in the research.

Sample size was determined with the help of statistical consultant based on Z formula and a confidence interval of 95 %, with 80 % power, type one error of 5 %, success in pain reduction (obtaining pain score of less than three during DwPT vaccine injection) of 41 % for MMR and then DwPT injection in the index pilot study. Thus with an effect size (difference in frequency of success in pain reduction between the two groups) of 30 %, the primary outcome was to be assessed in 33 children in each group. Finally, 35 children in each group were recruited to account for missing data and dropouts.

Eligible participants included children who aged 18 mo, without systemic illness and were in a healthy medical condition, accompanied by the mother and were referred for routine vaccination to the primary health care center.

Exclusion criteria consisted of neurodevelopmental delay, severe congenital malformation, febrile illness, receiving sedative hypnotic or systemic analgesic drugs (acetaminophen, ibuprofen, *etc*.) within the past 24 h and crying before vaccine injection.

Developmental status of the children was assessed using the Denver II Developmental Screening Test by the pediatric neurology researcher. The trial used computer generated equal simple randomization by random numbers and allocation ratio was 1:1 for the two groups. Since, routes of vaccine injection were different, blinding of parents of the participants, the nurse of primary health care center, the Data collector and the outcome assessor, was not possible and data analysts were only kept blinded to the allocation. But, concealment was done by placing the vaccine order for each serially participating child in a numbered and sealed opaque envelope that was opened by the pediatric research neurologist immediately before vaccination. Randomization and concealment were done by a researcher who was not clinically involved in the trial.

The children were randomly distributed into two groups. In group I, first intramuscular DwPT and then subcutaneous MMR vaccines were injected and in group II, children received subcutaneous injection of MMR first and intramuscular injection of DwPT vaccines, thereafter. The children were awake, quiet and alert and had clean diapers at the time of vaccines injection. Mothers of the children were in the vaccination room and held their children during the injection of vaccines.

DwPT and MMR vaccines of research were products of Pasteur Institute of Iran and in all of the children, vaccines were injected in similar conditions, by similar needles and by a trained nurse of primary health care center staff.

Dosage of the DwPT vaccine was 0.5 ml which was injected while the child was held by his or her mother with a 23 mm gauge needle into the vastus lateralis muscle, at a 90° angle with steady pressure, no aspiration, rapid injection of vaccine over 1–2 s and rapid withdrawal of the needle [6].

Dosage of the MMR vaccine was 0.5 ml which was injected subcutaneously with a 27 mm gauge needle into the fatty tissue over the triceps muscle, at a 45° angle.

Second vaccine was injected after 2 min from the first one.

The intervention was delivered by a nurse and primary and secondary outcomes were assessed by the pediatric resident of research. Paracetamol was not given before or after injections.

Primary outcomes included baseline pain score 5 s before the first vaccine injection, pain score during DwPT vaccine injection, pain score during MMR vaccine injection, overall pain score of vaccination and obtaining pain score of less than three during DwPT vaccine injection (success in pain reduction or painless vaccination). Pain score, when the needle was inserted to the skin, was assessed based on Modified Behavioral Pain Scale (MBPS). Parameters of MBPS which are presented in Table 1, included facial expression, crying

Table 1 Modified behavioral pain scale (MBPS)

	Parameters		
Scoring	Facial expression	Crying	Body movements
0	Definite positive expression (smiling)	Laughing or giggling	Usual movements and activity or resting and relaxed
1	Neutral expression	Not crying	-
2	Slightly negative expression (grimace)	Moaning, quiet vocalizing, gentle or whimpering cry	Partial movement (squirming, arching limb, tensing, clenching) or attempt to avoid pain by withdrawing the limb where puncture is done
3	Definite negative expression (furrowed brow eyes closed tightly)	Full lunged cry or sobbing	Agitation with complex/generalized movements involving the head torso or other limbs or rigidity
4	-	Full lunged cry more than baseline cry (scored only if child was crying at baseline)	-

and body movements by summing the score of each item and total score range of 0–10 [7]. In this study, the total pain score was calculated by pain score summation during DwPT and MMR injections and the sum of the two was considered as overall pain score of vaccination. However, in Ipp et al. study, pain score was assessed within 15 s after injection of second vaccine and it was considered as overall pain score [8].

Obtaining a pain score of less than three based on MBPS during the needle insertion into the skin was considered as success in reducing pain.

Secondary outcome was the duration of the baby crying during DwPT vaccine injection which was calculated from the onset of crying (when the needle was inserted) until crying was stopped after 5 s.

The data were analyzed using Statistical Package for the Social Sciences version 17 (SPSS, Chicago, Illinois, USA) statistical software. Recorded data were assessed for normal distribution using the Kolmogorov-Smirnov test and Chi-square test was used for data analysis of categorical variables and continuous and mean variables were compared using independent t-test between the two groups. Differences were considered significant at P values of less than 0.05.

Informed consent was taken from parents of the children before enrolling and this study has been approved by the

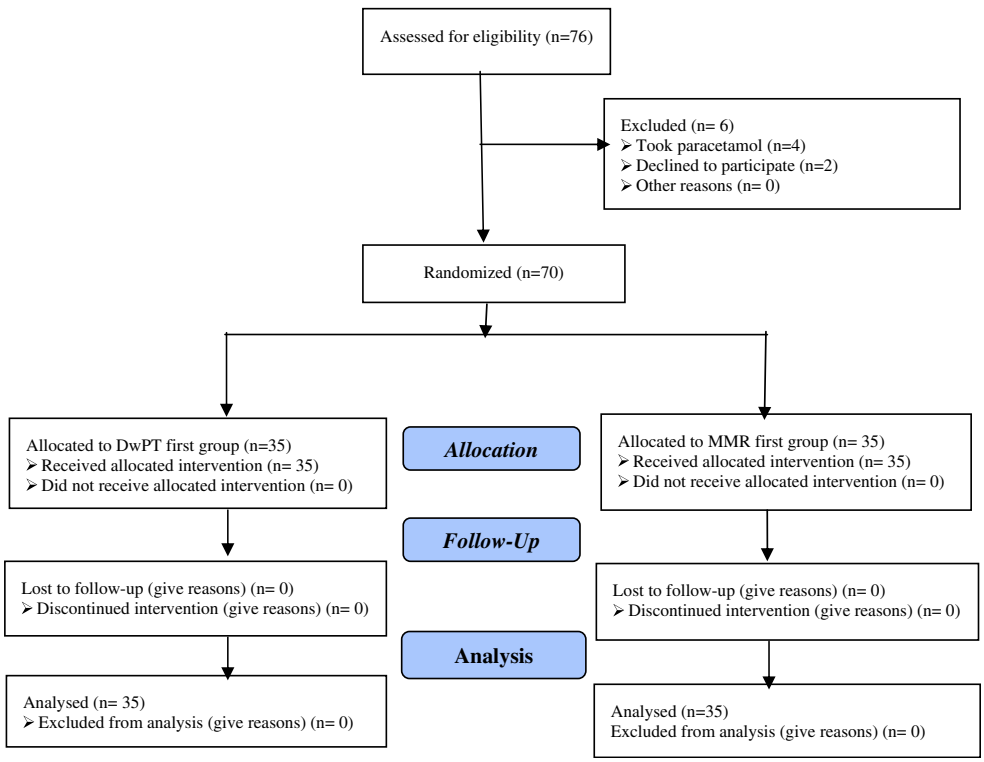

Fig. 1 CONSORT flow diagram

Table 2 Comparison of demographic characteristics of children in both groups

Data	Order of vaccine injection		*P* value
	DwPT and MMR	MMR and DwPT	
Sex			
Girl	16	21	0.2
Boy	19	14	
Weight in kg (mean ± SD)	9.53 ± 1.42	9.69 ± 1.46	0.6
Height in cm (mean ± SD)	79.54 ± 1.94	80.23 ± 2.92	0.2

Ethics Committee of Shahid Sadoughi University of Medical Sciences, Yazd, Iran. This research is registered in Iranian clinical trials by the address of www.irct.ir under registration number: IRCT201409042639N14.

Results

The design and conduct of this trial were straightforward, and authors did not have any losses or exclusions from the analysis and finally, 37 girls and 33 boys were evaluated in the two groups (Fig. 1). By Kolmogorov-Smirnov test, the data had normal distribution.

Comparison of demographic characteristics of the children in the two groups is shown in Table 2 which indicates that no statistically significant differences were seen from viewpoints of sex distribution, mean of weight and mean of height.

Table 3 shows comparison of pain scores in the two groups and indicates that the baseline pain score was 5 s before the first vaccine injection, pain score during DwPT vaccine injection and pain score during MMR vaccine injection were not statistically and significantly different in both groups. But, overall pain score of vaccination was lower when subcutaneous injection of MMR vaccine was done before intramuscular injection of DwPT vaccine.

Comparison of frequency of success in reducing pain (obtaining pain score of less than three during DwPT vaccine injection) and the duration of the baby crying during DwPT vaccine injection are presented in Table 4. It shows that success rate in pain reduction and crying duration were not statistically and significantly different in the two groups.

Table 4 Comparison of frequency of success in pain reduction (obtaining pain score <3) during DPT injection and crying duration during DPT injection in both groups

Data	Order of vaccine injection		*P* value
	DwPT and MMR	MMR and DwPT	
Success in pain reduction			
Yes	4	6	0.4
No	31	29	
Duration of baby crying in seconds (mean ± SD)	34.57 ± 15.15	33.41 ± 14.34	0.8

Discussion

Vaccination pain can be decreased through three 'P' approaches of pharmacological, physical and psychological methods [5]. Recommendations, such as using the least painful formulation of a vaccine, making the child sit up (or holding an infant), skin stroking or pressure application close to the site of injection before and during the injection, first injection of the least painful vaccine when two vaccines are being administered subsequently during a single visit and a rapid intramuscular injection performance without aspiration, have been suggested [9].

Basic understanding says, if more painful injection is given first, it is likely to affect the calculated pain score during the second injection (as the effect of first injection still persists) [9].

In the present study, the researchers have calculated the pain score for 5 s during the first injection and during the second injection. Based on the result, pain of intramuscular injection was more than that of subcutaneous injection in both groups and in simultaneous and concurrent injection of intramuscular DwPT and subcutaneous MMR vaccines at the same visit in 18-mo-old healthy children, vaccine administration order (intramuscular first and then subcutaneous or subcutaneous first and intramuscular thereafter) had no effect on intramuscular vaccine injection pain score which

Table 3 Comparison of pain scores in the two groups

Data	Order of vaccine injection		*P* value
	DwPT and MMR	MMR and DwPT	
Pain score before vaccination (mean ± SD)	2.26 ± 0.44	2.21 ± 0.41	0.5
Pain score during DPT vaccine injection (mean ± SD)	8.83 ± 1.59	8.17 ± 1.65	0.5
Pain score during MMR vaccine injection (mean ± SD)	7.71 ± 2.31	6.88 ± 2.44	0.4
Overall pain score of vaccination	15.61 ± 2.65	14.23 ± 1.35	0.04

was measured by Modified Behavioral Pain Scale (MBPS), and individual pain scores were not significantly different but the total was better when subcutaneous injection was given first.

In a study in Spain, injection order of two intramuscular vaccines (Pentavac: diphtheria, polio, tetanus, acellular pertussis and B Type Haemophilus influenzae) and meningitis C vaccine (NeisVacC) in 2 mo old healthy infants were evaluated and the authors concluded that average pain scores that were measured by MBPS, decreased when Pentavac was injected first and NeisVacC thereafter [10].

In a study in Karnataka, India, vaccine administration order of intradermal Bacille Calmette-Guérin (BCG) and intramuscular Hepatitis-B vaccines in term neonates were evaluated and the result showed that overall pain scores which were measured by the Neonatal Infant Pain Scale was lower when BCG vaccine was injected first and Hepatitis-B vaccine thereafter [11].

Ipp et al. in a study in Toronto, Canada, evaluated the effect of injection order of two intramuscular vaccines of diphtheria, polio, tetanus, acellular pertussis and Haemophilus influenzae type b (DPTaP-Hib) and the pneumococcal conjugate vaccine (PCV) on overall pain score that was measured by MBPS in 2–6 mo old healthy infants and it was concluded that overall pain score (pain score within 15 s after injection of second vaccine) decreased when DPTaP-Hib was injected first and PCV thereafter [8].

Possible explanation for this discrepancy may be related to different vaccines, different routes of injection and different time of assessment of overall vaccination pain score. If parameters such as pain score of 2 min after second injection had been measured in the present study, then possibly, total pain scores would not have been different as well.

Since, intramuscular vaccines are more painful than subcutaneous ones, administering the more painful injection first, causes the infant to focus attention on the procedure and the central and peripheral mechanisms of pain processing are activated, which together result in amplification of the pain signal during subsequent injections which are done immediately thereafter [8].

In conclusion, since the determination of vaccine administration order is a simple, effective, cost effective, and easy strategy in reduction of immunization pain and based on the result of the present study, overall pain score of vaccination in multiple vaccine injections at the same visit can be reduced when subcutaneous vaccine is injected before intramuscular one, it is worth to do other clinical trials with larger sample sizes for definition of vaccination order in simultaneous and concurrent injection of intramuscular and subcutaneous vaccines.

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Contributions All the authors contributed in this research and article preparation. RF will act as guarantor for the paper.

Compliance with Ethical Standards

Conflict of Interest None.

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