Comparative Evaluation of Pain Related Behavior During Administration of Local Anesthesia Using the Conventional Syringe and the Computerized Controlled Local Anesthetic Device in Children Exhibiting Different Levels of Anxiety: In vivo Study

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Authors’ contributions

This study was carried out under the supervision of author VB who also analysed the results. The conceptualization, designing and manuscript preparation was carried out by authors PGM and AM edited and carried out the literature review and critical analysis of the manuscript.

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ABSTRACT

Background: Pain experienced during dental procedures evolves into negative attitude towards dentistry, which is projected as anxiety and fear of the child. Administration of local anesthesia (L.A) although an intervention to render painless procedures, in itself causes pain by its conveyance. Wand® (Milestone Scientific, Livingston, NJ, USA) is a system introduced to decrease soreness in the course of anesthetic conveyance. Owing to the sparse information comparing conventional systems with those of Wand, the present study evaluated pain related behavior in children exhibiting pre-assessed variant levels of anxiety midst administration of local anesthesia.

Aim: To correlate pain related behavior amidst delivery of local anesthesia using conventional syringe and the Wand® STA system in children exhibiting variant anxiety levels.

Methodology: The In vivo study embodied 32 children aged between 6 to 8 years. The volunteered participants were pre-categorized into low and high anxiety using the Modified Child

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Dental Anxiety Scale. They were further divided using the SNOSE technique – receiving L.A the conventional syringe (group A) and those receiving L.A using the C.C.L.A.D (group B). Pain reactions and perceptions to the two delivery methods were calculated using subjective scales and objective physiological parameters.

**Results:** Comparing pain perception in pre-assessed anxiety categories affirmed a remarkable difference with the use of C.C.L.A.D and appeared to bestow effortless, pain-free delivery over the traditional cartridge delivery.

**Conclusion:** C.C.L.A.D builds a refined acclivity for dispersal of the solution, ergo an utilitarian tool in pediatric dentistry.

**Keywords:** Pain dental treatment computer controlled local anaesthetic device (C.C.L.A.D); wand single tooth anaesthesia (Wand® – STA); conventional, syringe; modified child dental anxiety scale (M.C.D.A.S); face, legs, activity; cry and consolability scale (F.L.A.C.C); visual analogue scale (V.A.S); oxygen saturation (SpO2); pulse rate (P.R).

**ABBREVIATIONS**

C.C.L.A.D : Computer Controlled Local Anaesthetic Device
S.T.A : Single Tooth Anaesthesia
D.P.S : Dynamic Pressure Sensing
Con : Conventional
L.A : Local Anaesthesia
CDS : Computerized Delivery System
M.C.D.A.S : Modified Child Dental Anxiety Scale
LA : Low anxiety
HA : High anxiety
Ml : Millilitre
Mm : Millimeter
SNOSE : Sequentially Numbered, Opaque, Sealed Envelope
ml/sec : Millilitre per second
% : Percent
F.L.A.C.C : Face, Legs, Activity, Cry and Consolability scale
V.A.S : Visual Analogue Scale
SpO2 : Oxygen saturation
PR : Pulse rate / Heart rate
MS Excel : Microsoft Excel
SPSS : Statistical package for the social sciences
< : Greater than
AMSA : Anterior and Middle Superior Alveolar

1. **INTRODUCTION**

Pain, an odious perceptible sentiment induced by a pernicious-stimuli is a convoluted and multidimensional happening that vitalizes the entity, heedless of factual or ostensible impairment, to interpose in mitigating its existence [1,2]. During dental procedures, pain is one of the major factors that have a lasting and profound impact on the behavior of a child. Pain encompasses one’s level of apprehension, belief, perception and disposition over the sore impetus [3]. In pediatric population, the developmental magnitude of corporeal and cerebral arrangement also impacts the pain response which is modulated by all contextual, psychological and physiological factors. Pain experienced during dental procedures leads to the development of negative attitude towards dentistry, which is projected as anxiety and fear in follow-up visits of the child. Hence, a pediatric dentist should focus on minimizing and controlling pain as it is an important aspect of successful treatment. Anxiety, a diversification of fear, is experienced in anticipation of a threatening stimulus and is accepted as a taxing facet for providing optimal patient care. Children usually dislike dental treatment owing to their fright and consternation allied especially to the use of injection. A relaxed and mollified child in a habitat that grants intricate dental therapy under anesthesia to be rendered devoid of imposing detrimental trauma is important for attaining clinical success [2]. Administration of local anesthesia (LA) is one such intervention obligatory in most dental treatments in order to render painless procedures. But local anesthesia conveyance is by injections which in itself causes pain to the patients. Pain experienced by the patient during an injection is twofold [4,2]. Firstly, mucosal piercing resulting in tissue damage and second, by the increase in pressure during infiltration of anesthetizing agent. Approaches such as behavioral modulation techniques (reframing, distraction) and also different painless delivery techniques like anesthetic-patches or gel, have been invented and attempted to ease the discomfort. However, these approaches have not been able to totally eliminate the pain experienced while injecting.

Wand® (Milestone Scientific, Livingston, NJ, USA) is a system introduced to decrease
soreness in the course of anesthetic conveyance. This computer-automated device, was first introduced in 1997 [5,2]. Milestone Scientific in 2007, came up with its variant Wand® STA (Single-Tooth-Anesthesia) [6]. Key to the development of this system was based on the fact of delivering anesthetic solution at a continual rate, dynamic pressure sensing, and volume thereby reducing the pain of inoculation, regardless of tissue resistance. It is known to anaesthetize the area promptly, ensuing an unnoticeable injection with an improved patient convenience, ease, decreased pain perception and consequently perturbation of injection [7].

Various studies have been conducted using the Wand to assess its potency, alone and as opposed to all the other available local anesthetic delivery systems. With a few studies showing lower pain ratings for computer mechanized injections as compared to injections using traditional syringe, to a few studies reporting similar pain ratings for needle insertion but decreased scores whilst depositing the anodyne using Wand have been observed [8,9,10]. In fact a few, with no difference between the two injection methods have also been reported [11]. It can be hypothesized that the differences can be attributed to different injector preferences for anxious and non-anxious children as any dental procedure is bound to cause some amount of apprehension in a child. Moreover, the anticipated reasoning for the variation in the findings could be a result of every individual’s own pain perception. Sequentially resulting in probable changes in the physiological parameter and one’s overall behavior. Therefore, before evaluating any of the aforementioned delivery approaches, it is paramount to pre-monitor every child’s level of anxiety.

Owing to the sparse information comparing conventional delivery systems with those of Wand in children with pre-assessed levels of anxiety, there is a need for further investigation in this area. Fear and anxiety causing dental procedures, not only result in disruptive behavior in a clinical set up but also increase the amount of perceived pain and with subsequent compromise in respiration and cardiac changes. Innumerable pain calibrations exist with particular indications, many of which are dependable and advocated. Pain can be self-appreciated by children six years of age and above. However, in those below six years of age, behavioral ratings are essential to estimate pain [12]. Only visual observation is unpredictable in accurately determining the adequacy of oxygenation and thus vitals should also be kept under check. Thus, anxiety and pain related behavior must be monitored and measured both objectively and subjectively.

The present study is hence a pioneer to evaluate pain related behavior in children exhibiting pre-assessed variant levels of anxiety during dental visits midst administration of local anesthesia using the conventional syringe and the computerized controlled local anesthetic device.

2. MATERIALS AND METHODS

This In vivo study was conducted on 32 children aged between 6 – 8 years reporting to the Out-Patient Department of Department of Paediatric and Preventive Dentistry at Dr. D.Y Patil Dental College and Hospital, Pune, India. Patients requiring dental treatment under local anaesthesia in the maxillary arch, with no previous dental experiences, exposure or contraindication to administration of local anaesthesia were included after obtaining a written informed consent from parents and an assent from the child. Medically compromised or children with suspected developmental delay were excluded from the study.

Before starting the treatment, child’s anxiety was recorded using pre-validated Modified Child Dental Anxiety Scale. Children representing trait anxiety under dental situation with scores less than 24 define category I (n = 16) of low dental anxiety and children with scores of 24 and higher define category II (n = 16) of high dental anxiety.13 Participants from each category were further randomly assigned to the following 2 groups – those receiving local anaesthetic administration using either the conventional cartridge syringe or the C.C.L.A.D (Wand® S.T.A system) using the SNOSE technique. (Table 1) In order to avoid bias, the delivery systems were not readily demonstrated to the subjects.

All patients from both the respective categories and groups, received maxillary infiltration and direct palatal injection with 30gauge needles and cartridges of 2% lignocaine (Lignospan special, Septodont) with delivery of 1 ml of anaesthetic agent buccally. A palatal infiltration was also dispensed on the palatal verge equidistant to the sulcular epithelium and the central line until slight blanching was noticed.
Table 1. Sample distribution in each group

<table>
<thead>
<tr>
<th>Category (Anxiety Levels)</th>
<th>Groups (L.A technique)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group A: Conventional technique</td>
</tr>
<tr>
<td>Category I: Low Anxiety (LA)</td>
<td>8</td>
</tr>
<tr>
<td>Category II: High Anxiety (HA)</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>16</td>
</tr>
</tbody>
</table>

In group B of both anxiety variants, the control unit of Wand® S.T.A (Wand Dental, Inc. Livingston, NJ, USA), was pre-programmed to S.T.A mode (speed mode 0.005 ml/sec) for both buccal and palatal infiltrations. With respect to use of Wand, the bevel of the needle was placed flat against the tissue and a dribble of anaesthetic solution was unloaded at once, ahead of the needle puncturing the tissue. Subsequently (4-5 seconds), the tip was progressed apically and a supplementary volume was administered to each root, allowing an anaesthetic trail to mature antecedent to tissue penetration. Getting to the level of the bony palate, dilatory release was pursued until diminutive whitening of adjoining area was visualized.

Physiological parameters (pulse rate and SpO2) objectively monitored anxiety and pain response with the help of pulse oximetry and the pain scores were measured and monitored using observational F.L.A.C.C (Face, Legs, Activity, Cry and Consolability) and V.A.S (Visual Analogue Scale) for subjective evaluation. The collected values were further tabulated and statistically analysed [13,14].

2.1 Statistical Analysis

After tabulation of data onto a database using MS Excel (Microsoft Corporation, Redmond, WA, USA), the statistical analysis was conducted using SPSS v. 19.0 (SPSS Inc., Chicago, IL, USA) with a significance level set at p < 0.05. Inter-group variables were analysed using unpaired-t test and the qualitative data was analysed with the Mann-Whitney U test.

3. RESULTS AND DISCUSSION

Both the categories of low and high dental anxiety were allocated into 2 groups and pain related behaviour was monitored using subjective evaluation and objective physiological parameters at both baseline and after administration of local anaesthesia.

Comparison of mean score of baseline pulse rate and oxygen saturation between conventional and C.C.L.A.D groups was found to be statistically insignificant implying a uniform distribution.

On comparing mean score of procedural pulse rate between conventional and C.C.L.A.D groups, a statistically significant difference was recorded (t = 2.203, p = .05) (Table 2). However, on comparing mean score of procedural oxygen saturation between conventional and C.C.L.A.D groups, results were found to be statistically insignificant. Similarly, comparison of pain related behaviour using F.L.A.C.C and V.A.S was insignificant in the subjects belonging to the low anxiety category.

An insignificant difference was seen on comparison of mean score of baseline pulse rate and oxygen saturation between conventional and C.C.L.A.D groups, thereby suggesting uniform distribution. On comparing the mean score of procedural pulse rate between conventional and C.C.L.A.D groups, a statistically significant difference was recorded (t = 3.199, p = .006) (Table 3). However, an insignificance was seen with respect to procedural oxygen saturation between conventional and C.C.L.A.D groups.

A statistically significant difference (Mann-Whitney U = 0.00, p = .001) was seen in comparison of pain related behaviour using F.L.A.C.C and V.A.S between conventional to the C.C.L.A.D groups in the subjects belonging to the high anxiety category.

3.1 Discussion

The level of anxiety is a principal factor in the response of a child to delivery of local
## Table 2. Distribution and comparison of pulse rate and oxygen saturation (baseline and procedural) along with comparison of pain related behaviour using F.L.A.C.C and V.A.S between conventional and C.C.L.A.D groups in low anxiety category

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>Mean Rank</th>
<th>Std. Deviation</th>
<th>Mean Diff</th>
<th>t</th>
<th>Mann-Whitney U</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR Baseline</td>
<td>IA: Conventional: LA</td>
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<td>100.7500</td>
<td>3.53553</td>
<td>.87500</td>
<td>.541</td>
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<tr>
<td></td>
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<td>.50000</td>
<td>.764</td>
<td>0.46</td>
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<td></td>
</tr>
<tr>
<td>SpO2 Baseline</td>
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<td>1.19523</td>
<td>.50000</td>
<td>.764</td>
<td>0.46</td>
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</tr>
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<td>.50000</td>
<td>.764</td>
<td>0.46</td>
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<td>1.03510</td>
<td>.50000</td>
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<td>0.46</td>
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<td>F.L.A.C.C</td>
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<tr>
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<td>21.500</td>
<td>0.26</td>
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<tr>
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<td>IB: C.C.L.A.D: LA</td>
<td>8</td>
<td>7.19</td>
<td>0.001</td>
<td>0.74</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

## Table 3. Distribution and comparison of pulse rate and oxygen saturation (baseline and procedural) along with comparison of pain related behaviour using F.L.A.C.C and V.A.S between conventional and C.C.L.A.D groups in high anxiety category

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>Mean Rank</th>
<th>Std. Deviation</th>
<th>Mean Diff</th>
<th>t</th>
<th>Mann-Whitney U</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
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<td>.849</td>
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<tr>
<td></td>
<td>IIB: C.C.L.A.D: HA</td>
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<td>.849</td>
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<td>.764</td>
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<tr>
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<td>.893</td>
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<tr>
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<tr>
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<tr>
<td>F.L.A.C.C</td>
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<td>12.50</td>
<td>0.00</td>
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</tr>
<tr>
<td></td>
<td>IIB: C.C.L.A.D: HA</td>
<td>8</td>
<td>4.50</td>
<td>0.00</td>
<td>0.001</td>
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<tr>
<td>V.A.S Procedural</td>
<td>IIA: Conventional: HA</td>
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<td>12.50</td>
<td>0.00</td>
<td>0.001</td>
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<tr>
<td></td>
<td>IIB: C.C.L.A.D: HA</td>
<td>8</td>
<td>4.50</td>
<td>0.00</td>
<td>0.001</td>
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</table>
anaesthetic fluid. A greater pain response and associated behavioural distress has been highlighted in anxious children to dental injections as compared to non-anxious children. Versloot et al. 2008, conducted a study comparing pain and distress response in children with dental injections and observed anxious children to have a more disruptive behaviour [11]. Various methods and computerized anaesthetic systems have been used and marketed to eliminate and/or minimize pain during injections. Wand, first of the C.C.L.A.D devices, introduced in 1997 has gained popularity [2]. Another variant, the Wand® Single Tooth Anaesthesia (S.T.A) was later launched by Milestone Scientific in 2006. Owing to the strictly regulated infusion pressure, a sizeable proportion of the agent can be administered with greater ease. Maintaining an ideal flow rate of anaesthetic solution along with a recommended bi-facial gyration of the tip during insertion is probably a major factor in achieving a comfortable anaesthetic injection [15].

Pain response to the aforementioned using several injection sites has been the subject of comparison in many studies since 1999. However, the attainable repercussion of pre-assessed consternation on pain and its perception has not been contemplated in studies comparing Wand with the conventional syringe. The intention of this study was thus to estimate and collate pain related behaviour in the course of administering local anaesthesia using conventional syringe and the computerized controlled local anaesthetic device (WAND® S.T.A) in children exhibiting low and high levels of anxiety.

Children were gauged using the Modified Child Dental Anxiety Scale (M.C.D.A.S) and split into categories of low (category I) and high (category II) anxiety. Children in these categories are within the concrete operational period of Jean Piaget’s cognitive theory and are proficient of reporting the differences in pain perception owing to their adult like cognitive adroitness and rational reasoning. Questioning children about dental anxiety is persuasively effortless, although verbal methods employed with very young children can be problematic for comprehension and intellectual ability [13]. Thus, M.C.D.A.S system was elected as it provides a uniform and understandable method of answering with a more detailed profile of the child’s specific response to dental procedures and sufficiently brief usage in a clinical setting. Although, self-affirming is regarded as the gold-standard in estimating of pain, subjective gauging may vary in opinion of one’s pain threshold level and thus, the use of at least two different scales like an amalgamation of self – claiming and observational parameter is firmly commended in young children [12,13]. In our study, pulse rate and oxygen saturation measurement were the physiological parameters used for evaluation as it contributes as an in-direct measure of pain and is unconditional to viewer’s bigotry, yielding salient authentication to undeviating observational parameters. Determination of oxygen saturation was done because it serves as a reliable physiological indicator. Also, not many studies taxied the relationship between anxiety, pain response and oxygen saturation. A drop in the oxygen saturation below normal (95-100%) measured as SpO2 on the pulse oximeter strikes a chord for discrepancy attributed to dissimilar pain threshold and physical reaction to a stimulus for every child [16].

Children tend to show increased muscle tension, verbal protest and crying when anxious. Anxious children, over - predict dental pain and are therefore more apprehensive [17,18]. Sensitisation prior to frame of reference to a paedodontist or presentation to concrete cure (Kuscu and Akyuz, 2008; Versloot et al., 2008), the trivial anticipation of pain and intrusion into the oral cavity are factors equally contributing in lowering one’s pain threshold [1,12]. In the present work, participants were pre-monitored and categorized into low and high anxiety. Amongst children manifesting contrasting magnitudes of anxiety, children presenting with low anxiety, noted a higher pulse rate in those administered local anaesthesia with the conventional syringe as compared to C.C.L.A.D. This may be due to the pain caused by the raised and unconstrained injection fluid pressure while using conventional syringe. A positive correlation between pressure at the start of injection and intensity of pain was also noted [19,20]. Our result, was alike to that of San Martin-Lopez et al., 2005 who illustrated an alteration in the norm pulse-rate on comparing the programmed device to cartridge syringe. [10] However, our results showed a remarkable difference to conclusions by Langthasa et al., 2012 who found no contraries in the physiologic specifications (pulse rate) betwixt the traditional cartridge and Wand inoculation [21].

With respect to subjective evaluation, the disruptive behaviour of the children was
insignificant in the low anxiety category, however, a significant increase was noted in the procedural pulse rate of highly anxious children receiving anaesthetic delivery using the conventional injection. Highly anxious children secured significantly higher scores in terms of pain-related response using the conventional system as compared to children treated using C.C.L.A.D. The above suggesting lesser coping strategies in highly anxious children over children with low anxiety towards dental treatment. This outcome was similar to that of Gibson et al., 2000; Allen K et al. 2002, who reported that C.C.L.A.D produced lesser disruptive behaviour with only a few children crying or exhibiting facial and body movements while receiving injection irrespective of the age group [22,23]. Ashkenazi et al. in 2005, compared the effectiveness of infiltration and intrasulcular injection, delivered by a computerized delivery system (CDS) and reported decreased stressful responses after both palatal and buccal inoculations using the CDS [24]. It was Al Amoudi N et al. in 2008, who evaluated the anaesthetic strength of the AMSA (Anterior and Middle Superior Alveolar) shot administered through C.C.L.A.D and concluded the computerized AMSA inoculation to be efficient in young adults. [25] Children’s feedback on affliction during C.C.L.A.D induced AMSA injection in contrast to conventional buccal/palatal injections were also evaluated by Feda M et al. in 2010. Statistically, reduced discomfort was reported with the C.C.L.A.D infused AMSA injection [20]. Thus, concluding the C.C.L.A.D to be a promising device.

From the present framework, having evaluated groups of low and high anxiety, no significant difference was noted in the pain perception using the conventional or the C.C.L.A.D. It was observed that children from both the groups experienced similar distress during both – traditional and computerised infiltration techniques. However, there was a hike in procedural pulse and oxygen saturation values on comparing procedural pulse rate in both conventional and C.C.L.A.D subgroups. Values although statistically significant, were not numerically and clinically considerable. Nevertheless, the results of our study, claim the superiority of C.C.L.A.D (WAND® S.T.A) to conventional cartridge injection technique in children as determined and demonstrated by lower distress related perception in the highly anxious children over children with lesser anxiety despite undergoing similar treatment in similar surroundings. The overall decline in pulse rate across the C.C.L.A.D groups in both the low and high anxiety categories, makes it better accepted than conventional system. As a matter of fact, Thoppe - Dhamodhara YK et al. 2015, proved C.C.L.A.D to produce reduced boisterous reaction in children by correlating anaesthetic delivery with physiologic values over subsequent visits [14]. Conflicting our study, were results by Asarch T et al. 1999, indicating computerized anaesthesia injection method was found to be comparable to the traditional method of anaesthesia injection. when comparing pain ratings and pain behaviour [8]. In fact, Ram; Peretz, 2003, also suggested no difference in the pain behaviour of children during the administration of local anaesthesia with a conventional injection or a computerized device when the operator was an experienced paediatric dentist [26] Similarly, Tahmassebi et al. 2009, compared the pain sensation on performing injections on children with Wand and traditional system and reported behavioural identicalness in both modes of delivery using V.A.S and Venham scale [27]. Another study by Kandiah P and Tahmassebi J. F in 2012, assessed pain experience comparing the outset of local anaesthesia (L.A) using the conventional cartridge versus the Wand computer-directed L.A. and no distinct soreness was reported at the inception of anaesthesia between the given groups [28]. However, the differences in the above and our results could have been attributed to the variant anxiety levels exhibited by children, wherein our study pre-assessed the anxiety levels in the entire study cohort. Further having analysed the results, there were no other major difference in variables such as age, gender and desired treatment for the tooth. Even though gender difference in dental fear and anxiety have been of increasing interest, no endeavour was made to match the gender in the various groups studied, which was in accordance to conclusion of Ram D; Peretz B., 2003 and Kandiah P.; Tahmassebi J. 2012, who suggest no distinction between girls and boys [26,28]. In our study, age varied across the different groups, irrespective of which, the use of C.C.L.A.D produced less pain ratings when compared to the traditional technique.

As our work was conducted in an academic setting, wherein children received treatment at low costs, the cost implications of Wand – the one of the assumed variables further influencing the result could not be studied. Another variable adding to the bias was not blindfolding the operator and participant whilst the procedure was
being conducted as it was thought to increase trepidation during dental procedures altering pain perception and eventually the physiological parameters, unlike older studies.

Despite having used various scales, measures and an independent observer for coding the behaviour as an attempt to minimize this bias, the limitations of the study cannot be overlooked. Even though, the quotient of this study has limited extrapolation, it is distinctive in children as it compares 2 delivery systems across children exhibiting pre-grouped levels of dental anxiety. The current study, thus recommends use of C.C.L.A.D as a suitable method of delivery of local anaesthesia towards achieving a relatively pain-free paediatric dental practice with significantly reduced disruptive behaviours in young adults who are habitually onerous and burdensome. The dental health team, by means of using the C.C.L.A.D can therefore, effectively and efficiently perform dental treatment for a child and at the same time instil a positive dental attitude and experience.

4. CONCLUSION

The current study gauged and collated pain related behavior during administration of local anesthesia using the conventional syringe and the computerized controlled local anesthetic device (Milestone – the WAND® S.T.A system) in children exhibiting differing levels of anxiety. Within the limitations of this study, we deduced that the computer-automated device of anesthesia, appeared to bestow effortless and pain-free delivery in opposition to traditional cartridge delivery in children with both low and high dental anxiety. However, supplementary experimentation, followed by scrutinized procurements of facts is essential to grade and classify the potency of computerized device at distinctive locations and sections of deciduous teeth in children.

DISCLAIMER

The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

All authors declare that written informed consent was obtained from the parent or caregiver before starting the procedure.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee (R and R Committee Approval from Dr. D. Y. Patil Dental College and Hospital, Pimpri, Pune, India: DPU/R & R(D) / 32 (26)/19) and have therefore been performed in accordance with the ethical standards laid down in the 1964 “Declaration of Helsinki.”

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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