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Abstract:

Background/Aim: Pulp necrosis is a frequent complication following dental trauma. The diagnosis of the state of the dental pulp can be challenging as most commonly used diagnostic tools are subjective and rely on a response from the patient, potentially making their use unreliable, especially in the child population. The aim of the study was to systematically review the evidence on the use of laser Doppler flowmetry in the assessment of the pulp status of permanent teeth compared to other sensibility and/or vitality tests. Methods: A systematic literature search, using MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials, www.clinicaltrials.gov and www.controlled-trials.com, in addition to citation and manual reference list searches, was conducted up to 15th January 2018. A risk of bias assessment was performed using the quality assessment for diagnostic accuracy studies tool (QUADAS-2) with all steps performed independently by two reviewers. Results: Four studies with a high risk of bias were included in the final analysis. Laser Doppler flowmetry was reported to be more accurate in differentiating between teeth with normal pulps and pulp necrosis with a sensitivity of (81.8-100%) and specificity of 100 % in comparison to other vitality tests such as pulp oximetry (sensitivity = 81.3 %, specificity = 94.9 %) and sensibility tests such as electric pulp testing (sensitivity = 63.3 – 91.5 %, specificity = 88 – 100 %). Conclusion: Despite the higher reported sensitivity and specificity of laser Doppler flowmetry in assessing pulp blood flow, these data are based on studies with a high level of bias and serious shortfalls in study designs. More research is needed to study the effect of different laser Doppler flowmetry's parameters on its diagnostic accuracy and the true cut-off ratios over which a tooth could be diagnosed as having a normal pulp.

Introduction

The prevalence of traumatic dental injuries is reported to be approximately 20% in children and adolescents with higher percentages reported in adults.[1] Pulp necrosis is one of the sequelae of traumatic dental injuries, which if not managed appropriately could lead to pain, and infection.[2] Therefore, accurate diagnosis of the pulp status of traumatised permanent teeth is an essential component in the management of dental injuries and long term survival of traumatised teeth.[3] Accurate pulp diagnosis is achieved through a combination of the patient history, clinical and radiographic assessments including the use of sensibility and/or vitality tests which are an integral part of the diagnostic process.[4]

Several diverse sensibility and vitality pulp tests are available. Sensibility is defined as the ability to respond to a stimulus. Sensibility tests offer an assessment of pulp health through the stimulation of pulp nerve fibres, therefore, relying on the patient's understanding and cooperation. On the other hand, vitality indicates the presence of blood supply within the tissues. Thus, vitality testing involves assessing the pulp's blood supply offering an objective approach to assessing pulp blood flow that is not reliant on the patient's understanding and response to stimuli.[5] Vitality tests include laser Doppler flowmetry (LDF), pulse oximetry and more recently the use of ultrasound Doppler flowmetry.[6,7]

LDF was first described in the dental literature in 1986.[8] The primary technique utilised a light beam originating from a helium–neon (He–Ne) laser emitting with a wavelength of 632.8 nm. Other laser wavelengths have since been used such as 780–820 nm. The laser light reaches the dental pulp from a fibre optic probe positioned against the tooth being assessed. When entering the tissues, the laser light is absorbed and scattered by the moving and circulating red blood cells. The photons that interact with red blood cells are Doppler–shifted according to the Doppler principle. The backscattered and returned light is then detected and

registered by a photodetector leading to a signal production. The unit used to record the scattered signals or "the concentration and velocity of cells " is termed Flux and assigned an arbitrary unit termed the perfusion unit (PU).[6]

The objectivity, non-reliance on patient's understanding and response, non-invasiveness and ability to test blood supply rather than sensation offers excellent advantages over pulp sensibility tests. The results of LDF, however, should be carefully interpreted due to the inability of the device to measure blood flow in absolute units, in addition to the non-linear relation between the signal output and blood flow rate.[9] Other drawbacks include signal contamination by gingival or periodontal blood supply, high equipment cost in comparison to other pulp tests and the need for patient cooperation as any movement of the apparatus or patient could affect the results.[10,11] Th aim of this review was to systematically assess the evidence from clinical studies on the use of LDF in assessing and monitoring the pulp status of permanent teeth compared to other sensibility and/or vitality tests.

Materials and Methods

The full research protocol was registered and published on PROSPERO, Centre for Reviews and Dissemination (CRD) at the University of York, UK (Registration details: CRD42016035457). A systematic electronic search, citation search and reference list screening were performed. The initial electronic databases search was performed on 2nd March 2016 and included MEDLINE (1946 to February week 3, 2016), EMBASE and EMBASE classic (1947 to 2nd March 2016) and Cochrane Central Register for Controlled Trials CENTRAL. In addition, a search for ongoing trials was conducted on two websites; www.clinicaltrials.gov and www.controlled-trials.com. Dissertation and thesis searches were performed using ProQuest while conference abstracts and proceedings were searched using BIOSIS database. The electronic search strategy was formulated under the supervision of a

specialist librarian (University of Leeds Library). The medical subject headings (MeSH) / keywords and the search strategy utilised for MEDLINE were as follows: (exp Dentistry OR Dent* OR exp tooth OR tooth* OR teeth* OR pulp* OR exp Dental pulp) AND (exp laser Doppler flowmetry OR Doppler* OR LDF*), with no limits used. The search strategy was adapted and applied to other databases. EndNote (X 7.4 Thomson Reuters) was used to manage references and remove duplicate records. The electronic search was repeated towards the end of the review process (15th January 2018).

The PICOS methodology was utilised in formulating the research question. The types of participants included were over the age of six years, participants with normal and necrotic pulps and studies where tooth vitality/sensibility had been followed up for at least six months. Types of intervention and comparators included vitality testing of permanent teeth using LDF compared to any type of vitality and/or sensibility tests.

Studies comparing healthy and necrotic pulps were included with the reference standards included a tooth with a pulp known to be normal with no clinical or radiographic signs or symptoms of loss of blood supply, in addition to no history of trauma, no caries nor any dental anomalies (composite reference standard). Moreover, a tooth known to have no pulp (such as pulp extirpated / root canal treated teeth).

Prognostic studies where LDF was used in assessing teeth with damaged and unknown pulp status such as traumatised teeth were also included. The reference standards for this type of studies were a composite reference standard which included signs of loss of blood supply including clinical signs of loss of blood supply and presence of infection in the root canal system such as abscess formation, sinus tract formation, tenderness to percussion / palpation, radiographic signs of periapical pathology, infection related resorption and hyperaemic dental pulp upon root canal treatment. Signs of a normal pulp included continuation of root

formation on radiographic views in teeth with immature root formation and none of the signs stated above for loss of blood supply.

Outcome measures were defined in accordance to published criteria for such studies.[12,13] The primary outcome measures included sensitivity, identifying necrotic and infected teeth as having a necrotic and infected pulp, and specificity, identifying normal teeth as having a normal pulp. Additionally, the secondary outcomes included positive predictive value, negative predictive value, repeatability, reproducibility, reliability and Flux ratio.

This systematic review included randomised controlled clinical studies, controlled trials, cross sectional studies including diagnostic cohort studies and diagnostic case-control studies. Prognostic or predictive studies were also included. Studies presented in English language only were included.

The exclusion criteria were participants under the age of six years, studies where primary outcomes of accuracy, sensitivity and specificity are not stated or not possible to calculate. Case series, case reports, reviews and in vitro studies were also excluded. Prognostic or predictive clinical studies with less than six months follow up were as well excluded.

Electronic searching was performed by one reviewer (N.G) while two reviewers (N.G and A.B) performed study selection, data collection and quality assessment. Any disagreement was resolved by consensus or consulting a third researcher (H.N). Articles meeting the inclusion criteria were selected for full text screening. The authors were contacted for additional information when necessary. A data extraction form was based on the Centre for Review and Dissemination guidance for undertaking reviews in health care. The form was piloted using one of the included studies.

The quality assessment tool used to evaluate the included studies was the QUADAS-2, which is recommended by the Cochrane collaboration, Agency for Healthcare Research and Quality, and the UK National Institute for Health and Clinical Excellence for use in systematic reviews of diagnostic accuracy studies. The QUADAS-2 tool assesses two aspects: risk of bias and applicability of concerns. These two aspects are assessed based on three domains: patient selection, index test and reference standard. In addition to these three domains, a fourth domain of flow and timing was also used for the assessment of risk of bias. All domains should be rated as low risk of bias and low concerns regarding applicably in order for a particular study to be rated as having a low risk of bias and applicability concerns.[14]. Piloting of the quality assessment process on one of the included studies was performed in order to calibrate and train both assessors.

Results

The total number of citations identified was 2890 (2569 at initial electronic search, 318 citations through final electronic search and 3 citations through reference list screening (Figure 1). After removal of duplicates (n = 784), 2106 potential eligible studies were identified. Following title and abstract screening, 2061 studies were excluded leaving 45 articles for full article assessment. Forty one studies were excluded leaving four studies to be included in the final qualitative assessment (Figure 1). [15-18] Although the outcome measures were not specified in one of the included studies, the study provided enough information to calculate the sensitivity and specificity of the tests, therefore, allowing it to be included.[18]

All included studies adopted a cross sectional diagnostic cohort design. Blinding and randomisation were not performed in any of the included studies. The participant's age range (Table 1) was very wide in three of the included studies (6.5-74 years),[15,17,18] while the fourth study included a narrow age range (12-18 years).[16]

There were large variations in LDF devices and techniques used in all included studies (Table 2). In terms of LDF device characteristics, there were variations in the laser wavelength used (780 nm was used in two studies, [15,16] while 632.8 nm was used in the other two studies [17,18]) and the probe characteristics (number of probes, fibre diameter and fibre separation) (Table 2).

In terms of LDF technique used, there were also differences in the duration of LDF measurements (20 seconds - 3 minutes) and the cut-off ratio used in identifying tooth vitality in all included studies (Table 2). An isolation splint was used in all studies; however, a rubber dam was not used in any study.

LDF showed a sensitivity of 81.8-100 % and specificity of 100 % in three studies.[16-18] LDF was compared to electric pulp testing (EPT) in three studies with EPT showing sensitivity and specificity of 63.3% – 91.5% and 88-100%, respectively.[16-18] LDF was compared to ethyl chloride in only one of the included studies, showing sensitivity and specificity of 92 % and 89 %, respectively.[17] Accuracy and repeatability of LDF in comparison to four other dental pulp tests were reported in the fourth study with a score of 96.3% and 65%, respectively.[15] Pulse oximetry was compared to LDF in one study showing lower sensitivity (81.3%) and specificity (94.9 %) to that of LDF (Table 3).[16]

The quality assessment showed a high level of bias in all included studies in terms of patient selection, index testing, reference standards, as well as flow and timing as shown in Figure 2.[15-18] With regards to applicability concerns, one study exhibited high concerns regarding applicability,[15] while three studies exhibited low concerns (Figure 2). [16-18]

Discussion

This systematic review focused on assessing the accuracy of LDF compared to all other sensibility and vitality tests in assessing the pulp status of permanent teeth. Four studies with high levels of bias were identified.[15-18]

Some of the principles or criteria assessed during quality assessment of the included studies were the use of reference standards and blinding. The reference standard is the best currently available tool in identifying a condition against which the index test (LDF) is evaluated. Selection of the reference standard plays a very critical role with regards the validity of a test accuracy study.[19] The reference standards used in the included studies, in order to identify a tooth with pulp necrosis as truly having a necrotic pulp, was root canal treatment in one study,[16] the presence of necrotic pulp or bleeding on pulp extirpation and root canal treatment in the other studies.[15,17,18] Bleeding following pulp extirpation is a subjective sign of pulp necrosis, therefore, should not be used as a reference standard. The reference standard for teeth with normal pulps was based on the lack of clinical and radiographic signs/symptoms of infection which is appropriate for such studies. Incorrect initial classification of the pulp status of the included teeth may result in over/under estimation of the dental pulp tests used.

Test review bias (blinding) occurs when results of the reference standard are known to the operator carrying out the diagnostic test while the test results are interpreted. The nature of the tests makes it hard to blind the examiner. However, the use of isolation splints with small windows showing teeth under assessment could allow blinding of the examiner to the pulp

status of the assessed teeth while using different sensibility/vitality tests. Interpretation of the diagnostic tests is usually influenced by the knowledge of the other tests or the condition of the teeth to be tested. Therefore, operator blinding of the examined tooth condition is mandatory in diagnostic accuracy studies.[20] This, however, was lacking in all included studies.

The studies included showed higher sensitivity and specificity of LDF compared to other sensitivity and vitality tests. However, the results of this systematic review highlight the inconsistencies and variabilities of the LDF machine's specifications (wavelength, probe specifications etc.) and application techniques (time of application, use of gingival shields etc.) used in assessing pulp blood flow. Such variability prevents comparison and synthesis of the LDF's published results. Factors such as the degree of LDF's laser penetration, gingival and periodontal signal contamination, the location of LDF's probe, the duration of the Flux measurement and the cut-off Flux value/threshold to which a tooth is considered to have no blood supply should be taken into consideration when using LDF and when planning and executing any future trial.

Laser penetration has been shown to be affected by crown restorations and crown colour change.[21,22] Therefore, inclusion of heavily restored teeth in studies might affect the LDF's accuracy. One of the studies included in this systematic review included heavily restored teeth and reported a high accuracy of LDF (96.3 %) in comparison to other dental pulp tests.[15] Such an effect should have been considered and reflected in the results of that study as Flux values might have been affected leading to misinterpretation of the results.

There were inconsistencies between the studies with regards to the duration of LDF measurement. It is well established that movement artefacts, whether related to the patient or apparatus itself, affect LDF readings. Therefore, allowing sufficient time for recording stable

Flux recording is recommended.[6] Including unstable movements' artefacts in the analysis may increase the Flux value leading to mis-interpretation of the results. Flux duration measurement ranged from 45 seconds,[16] to 3 minutes [17] in the included studies, with no reference to allowing stable Flux readings.

Another crucial factor in diagnostic accuracy studies is the use of a cut-off ratio/threshold (diseased pulp Flux/ known healthy pulp Flux) to aid the diagnosis. Ideally, a pre-specified threshold between a healthy tooth and a tooth with pulp necrosis must be established.[14] A pre-specified threshold was only mentioned in one of the studies included in this review with a cut-off ratio of 0.6 used (a ratio $\geq$ 0.6 (diseased/healthy) indicated a healthy pulp).[15] The authors based this ratio on the work of Ingolfsson et al,[18] which included in this review, and that of Roebuck et al, [23] which is not included in this review due to the lack of direct comparison with other sensibility/vitality tests.

LDF results of 11 pairs of healthy and necrotic pulps showing a significant lower Flux values for necrotic pulps in comparison to healthy pulps using four different probes have been reported in the study of Ingolfsson et al.[18] That study, however, showed spectrum bias, differences in disease severity, as four teeth were diagnosed with periapical radiolucencies, one tooth with submucosal abscess and one tooth with pulp canal obliteration. Teeth with such conditions should have been excluded as this could have caused inconsistencies in the accuracy estimates of the tests.

Roebuck et al assessed the effect of bandwidth filter, laser wavelength, fibre separation and probe position on the healthy/necrotic pulp ratios of Flux signals recorded from 11 healthy and non-endodontically treated teeth with pulp necrosis have been reported.[23] The combination of 633 nm with a 3 KHz bandwidth using a probe with a 500 μm placed 2-3 mm from the gingival margin was considered the most reliable combination. Moreover, a cut-off

ratio, used in determining pulp necrosis, was recommended if healthy pulps Flux / necrotic pulps Flux > 1.25 (a Flux ratio > 0.8 diseased/healthy) compared to the 0.6 reported.

Despite the limitations of these two studies, and indeed this systematic review, these studies highlighted the need for better quality diagnostic accuracy studies assessing the effect of different combinations of LDF parameters (such as wavelengths, probes used) on the cut-off ratio used in diagnosing pulp status before LDF could be recommended for clinical use.

Age related pulp changes could also contribute to changes in pulp blood flow, thus affecting Flux and Flux cut-off values. Such changes include higher pulp blood supply in immature teeth versus lower blood supply in calcified teeth or teeth with smaller pulp chambers due to secondary dentine formation.[24] There was a wide variation in age range in three included studies with the ages of the subjects ranging from 6.5-74 years.[15,17,18] More studies are recommended which should include a younger age group, where trauma occurs before root development is complete, as the assessment of pulp healing after trauma can be more challenging due to the child's anxiety often making routinely used sensibility tests less reliable.

Conclusion

Despite the higher reported sensitivity and specificity of LDF in assessing pulp vitality, these data are based on studies with high level of bias and serious shortfalls in study designs. This systematic review highlights inconsistencies in the evidence supporting the use of the LDF in assessing pulp vitality of permanent teeth. Further high quality diagnostic clinical trials are needed to determine LDF's true cut-off ratios over which a pulp could be diagnosed as necrotic. More research is also needed to study the effect of different LDF parameters on its diagnostic accuracy before such a tool, which is relatively expensive, could be reliably recommended for routine use in everyday practice.

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Legends to Tables

Table 1: A summary of the demographics and characteristics of the four included studies.

Table 2: A summary of LDF techniques used in the four included studies.

Table 3: A summary of the outcome measures reported for LDF in comparison to other sensibility and vitality tests as reported in the included studies.

Legends to Figures

Figure 1: Preferred Reporting Items for Systematic Reviews and Meta-Analyses flowchart summarising the systematic review process in the identification of included studies.

Figure 2: A tabular presentation of the results of the QUADAS-2 quality assessment of the studies included.

Study/Year	Study design	Sample size	Age	Teeth included	Disease characteristics	Comparators	Randomisation	Blinding	Reference test
Chen 2011 (13)	Cross sectional	20 patients ; 121 teeth	18-74	Maxillary and mandibular incisors, canines, premolars and molars.	Teeth suspected or known to have pulp pathosis or provisionally diagnosed as having a healthy pulp.	▪ CO_2 (carbon dioxide crystals) ▪ Ice ▪ Refrigerant spray (Endo Frost) ▪ Electric pulp testing	No	No	Root canal treatment
Karayilmaz 2011 (14)	Cross sectional	51 patients ; 59 pairs of anterior teeth	12-18	Maxillary central and lateral incisors	Endodontically treated teeth and healthy control teeth.	Electric pulp testing and Pulse oximetry	No	No	Clinical and radiographic examinations. Pulpless teeth had root canal treatment
Evans 1999 (15)	Cross sectional	Group 1: 57 patients ; 57 teeth with necrotic pulps and 53 healthy control teeth Group 2: 84 patients ; 84 vital teeth	6.5-33.5	Maxillary and mandibular anterior teeth	Teeth with healthy and necrotic pulps	Electric pulp testing and ethyl chloride	No	No	No clinical / radiographic signs or symptoms of infection for the healthy teeth No bleeding on pulp extirpation for non-vital teeth.
Ingolfsson 1994 (16)	Cross sectional	Group 1: 9 patients; 11 healthy teeth and 11 teeth with necrotic pulps. Group 2: 10 patients with 20 healthy teeth	11-37	Maxillary and mandibular anterior teeth.	Teeth with healthy and necrotic pulps	Electric pulp testing	No	No	Pulp necrosis was confirmed during root canal treatment. Healthy teeth tested positive to EPT , exhibited no discolouration and normal radiographic examination.

Study	Rubber dam used	Splint used	Location of probe	LDF device used and wavelength	Type of probe	Duration of LDF measurement	LDF cut-off ratio used	Unit of measurement
Chen 2011 (13)	No	Polyvinyl	2–3mm above the gingival margin	• MoorLAB/FloLAB; Moor Instruments Ltd, Axminster, UK. • Wavelength: 780 nm	• Double channel • 0.5-mm fibre separation.	90 seconds	Diseased pulp flux/ known healthy pulp flux ratio is less than or equal to 0.6	Flux
Karayilmaz 2010 (14)	No	Silicon-impression-based	2 mm above the gingival margin.	• BLF21A • Wavelength: 780 nm	• Single channel • Diameter: 1.5 mm, two fibres in 0.2 mm diameter centres 0.5 mm apart.	20 optimum seconds out of 45 seconds.	1/10 ratio between the pulp blood flow values measured by LDF	PU
Evans 1999 (15)	No	A two-stage green elastomeric splint.	Between 2 and 3 mm from the gingival margin.	• Perimed PF2b, Stockholm, Sweden. • Wavelength: 632.8 nm	• Single channel • Probe with 0.5 mm fibre separation.	3 min (where patient cooperation allowed)	LDF healthy pulp: flux equal or more than 7.0 PU and amplitude SWV equal or more than 1.6 PU LDF necrotic pulp : flux < 7.0 PU (determined post calculation and analysis not before) LDF Intermediate healthy pulp: flux equal or more than 7.0 PU but amplitude SWV < 1.6 PU	Two LDF signal variables were measured visually; Flux and SWV. Both measured in PU.
Ingolfsson 1994 (16)	No	Rubber base material	2-3 mm from the gingival margin.	• A Periflux PF3 laser,Perimed, Sweeden. • Wavelength: 632.8	• Double channel • Five probes used (fibre diameter/ fibre separation) mm o 0.2/1.5 o 0.2/1.0 o 0.2 / 0.8 o 0.2 / 0.5 o 0.125 / 0.25	1.5 to 2 minutes	No cut off used, only significance difference between readings.	Flux

PU: Perfusion unit PBF: Pulp blood flow SWV: amplitude of slow wave vasomotion.

Outcome measures	Tests	Chen 2011 (13)	Karayilmaz 2010 (14)	Evans 1999 (15)	Ingolfsson 1994 (16)
Sensitivity (%)	**LDF**	---	100	100	81.8 – 90
	EPT	---	91.5	87	63.3
	PO	---	81.3	---	---
	EC	---	---	92	---
Specificity (%)	**LDF**	---	100	100	100
	EPT	---	88	96	100
	PO	---	94.9	---	---
	EC	---	---	89	---
Positive predictive value (%)	**LDF**	---	---	---	100 (probe 125/250)
	EPT	---	88.5	---	100
	PO	---	94.1	---	---
Negative predictive value (%)	**LDF**	---	---	---	50 (probe 125/250)
	EPT	---	91.2	---	73
	PO	---	83.5	---	---
Accuracy (%)	**LDF**	96.3	---	---	---
	EPT	97.7	---	---	---
	CO2	97	---	---	---
	EF	90.7	---	---	---
	ICE	84.8	---	---	---
Repeatability	**LDF**	0.65	---	---	---
	EPT	0.43	---	---	---
	CO2	0.43	---	---	---
	EF	0.57	---	---	---
	ICE	0.67	---	---	---

EC: Ethyl chloride **EF: Endo Frost**