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NECROSE PULPAR ASSINTOMÁTICA EM DENTES HÍGIDOS DE INDIVÍDUOS COM ANEMIA FALCIFORME: INVESTIGANDO CAUSAS E NECESSIDADE DE TRATAMENTO

SÃO LUÍS

2018

CYRENE PIAZERA SILVA COSTA

NECROSE PULPAR ASSINTOMÁTICA EM DENTES HÍGIDOS DE INDIVÍDUOS COM ANEMIA FALCIFORME: INVESTIGANDO CAUSAS E NECESSIDADE DE TRATAMENTO

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Orientadora: Profa. Dra. Soraia de Fátima Carvalho Souza

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A Comissão julgadora da Defesa do Trabalho Final de Doutorado em Odontologia, em sessão pública realizada no dia 22/ 02/ 18, considerou a candidata.

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É melhor tentar e falhar, que preocupar-se e ver a vida passar.

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LISTA DE SÍMBOLOS

$\%SaO_2$ = Porcentagem de Saturação de Oxigênio

X= Média

χ^2= Teste do Qui-quadrado

LISTA DE ABREVIATURAS

ABEP= Associação Brasileira de Estudos e Pesquisa

AF= Anemia Falciforme

CEP= Comitê de Ética em Pesquisa

CFI= Comparative Fit Index

CFP= Carga Fatorial Padronizada

CFs= Crises Falcêmicas

CoM= Co-morbidades

CSE= Classe Sócio Econômica

DAG= Gráficos Acíclicos Direcionados

DM-2= Diabetes Mellitus tipo 2

Dp= Desvio Padrão

Fig= Figura

HbA= Hemoglobina A

HbF= Hemoglobina Fetal

HbS= Hemoglobina S

HbSS= Homozigose

HEMOMAR= Supervisão de Hematologia e Hemoterapia do Maranhão

HU= Hidroxiuréia

IC 90%= Intervalo de confiança 90%

IC 95% =Intervalo de confiança a 95%

MED= Medicação

MEE= Modelagem de Equações Estruturais

N° CFs/ano= Número de Crises falcêmicas no último Ano

NPA= Necrose Pulpar Assintomática

OP= Oximetria de Pulso adaptada a Odontologia

PCR= Polymerase Chain Reaction (Reação em Cadeia de Polimerase)

qPCR= PCR em tempo real

R^2= Coeficiente de correlação

RMSEA= Root Mean Square of Approximation

SCR = Sistema de Canais Radiculares

Tab= Tabela

TF= Traço Falciforme

TLI= Tucker-Lewis Index

TSF= Teste de Sensibilidade a Frio

WRMR= Weighted Root Mean Square Residual

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RESUMO

Introdução: Indivíduos com Anemia Falciforme (AF) podem apresentar dentes hígidos com Necrose Pulpar Assintomática (NPA), provavelmente devido a vasoclusão da circulação do órgão pulpar pelo acúmulo de hemácias falciformes, que morrem precocemente e se aderem com mais facilidade ao endotélio vascular. Desta forma, os objetivos do presente estudo foram explicar os mecanismos envolvidos na associação entre AF e NPA (primeiro estudo); e investigar a necessidade de tratamento endodôntico desses dentes (segundo estudo), por meio dos parâmetros clínicos, imagenológicos e microbiológicos.

Métodos: O primeiro estudo é do tipo coorte prospectiva e o segundo é transversal aninhado a coorte prospectiva. No primeiro estudo, a amostra foi constituída por 140 indivíduos com AF maiores de 16 anos, assistidos pela Supervisão de Hematologia e Hemoterapia do Maranhão (HEMOMAR), em São Luís, MA, Brasil. Foram incluídos sujeitos com pelo menos um dente com coroa hígida, ausência de histórico de parestesia do nervo alveolar inferior nos últimos seis meses, sem história prévia de traumatismo dentário e sem doença periodontal. O diagnóstico da NPA em dentes com coroas hígidas foi obtido por meio da porcentagem de saturação de oxigênio (%SaO_2) pulpar utilizando um oximetro de pulso adaptada à Odontologia (OP). A %SaO_2 corpórea e o número de Crises Falcêmicas no último ano (Nº CFs/ano) foram obtidos de cada indivíduo. Nº CFs/ano foi considerada variável contínua. As co-morbidades (CoM) foram consideradas variável latente formada pelos indicadores: presença de cardiopatias, alterações hepáticas, Diabetes mellitus tipo-2 e lesões osteoarticulares. O desfecho dicotômico foi ter NPA em dentes com coroas hígidas. Nº CFs/ano e %SaO_2 corpórea foram testadas como variáveis mediadoras da associação CoM-NPA. O modelo teórico proposto foi analisado por meio da Modelagem com Equações Estruturais (α=0.05). No segundo estudo, foram selecionados 10 indivíduos falcêmicos assistidos pela HEMOMAR que apresentaram pelo menos um dente com coroa hígida sem calcificação pulpar, e diagnóstico clínico de NPA por meio da OP e do teste de sensibilidade pulpar ao frio (n=27 dentes). Calcificações pulpares, alterações no periápice e ligamento periodontal foram identificadas em análises tomograficas. A detecção de bactérias foi realizada por meio de cultura bacteriana, fluorescência e do PCR (Polymerase Chain Reaction) em tempo real. As diferenças de prevalência de NPA entre as co-variáveis cor, sexo, idade e maxilares foram identificadas pelo teste exato de Fisher (α=0.05).

Resultados: No primeiro estudo, foram observados 125 indivíduos sem NPA e 15 com NPA. As CoM foram associadas à presença de NPA (CPF=1.061; p=0.028). Entretanto, essa associação não foi explicada pelo Nº CFs/ano (CPF=0.128; p=0.579), nem pela %SaO_2 corpórea (CPF=0.128; p=0.579). No segundo estudo, o diagnóstico clínico da NPA foi confirmado na maioria dos dentes (81.5%). A NPA foi mais prevalente nos maxilares superiores (p= 0.016). A quantidade de DNA bacteriano detectada foi inferior a 100 cópias/µL em apenas 18,5% da amostra. Os achados imagenológicos identificados em 18,5% dos dentes foram: espessamento do ligamento periodontal ≤ 0,5 mm (n=3), hipercementose (n=1) e nódulo pulpar (n=1).

Conclusão: Concluiu-se que a presença de CoM em indivíduos falcêmicos, principalmente as lesões osteoarticulares, aumenta a prevalência de NPA em dentes hígidos (primeiro estudo). Não há necessidade do tratamento endodôntico imediato nestes dentes, mas recomenda-se a proservação periódica clínica e imagenológica como protocolo de prevenção de infecção nestes indivíduos (segundo estudo).

Palavras-chave: Anemia Falciforme. Necrose Pulpar Dental. Oximetria de Pulso. Microbiologia.

ABSTRACT

Introduction: Individuals with sickle cell anemia (SCA) may present with healthy teeth with asymptomatic pulp necrosis (APN), probably due to the vasoconstriction of the pulpal organ circulation due to the accumulation of sickle cells, which die early and adhere more easily to the vascular endothelium. In this way, the objectives of the present study were informed about the processes involved in the association between SCA and APN (first study); and to investigate the need for endodontic treatment of these teeth (second study), through clinical, imaging and microbiological parameters.

Methods: Both studies were of the transverse type nested to a retrospective cohort. In the first study, the sample consisted of 140 individuals over the age of 16, assisted by the Hematology and Haemotherapy Supervision of Maranhão (HEMOMAR), in São Luís, MA, Brazil. Patients are subjects with at least one tooth with a healthy crown, absence of history of paresthesia of the inferior alveolar nerve in the last six months, without previous history of dental trauma and without periodontal disease. The diagnosis of APN in teeth with healthy crowns was obtained through pulsatile oxygen saturation percentage (%SaO2) using a pulse oximeter adapted to Odontology (PO). The body %SaO2 and the number of sickle cell crises in the last year (No. SCCs/year) were obtained from each individual. No. SCCs/year was considered a continuous variable. Co-morbidities (CoM) were considered as latent variables formed by the following indicators: presence of heart diseases, hepatic, type 2 diabetes mellitus and osteoarticular lesions. The dichotomous outcome for APN in teeth with healthy crowns. No. SCCs/year and of body %SaO2 were tested as mediator variables of the CoM-APN association. The model was analyzed through Modeling with Structural Equations complemented by Aciclic Directed Charts (α=0.05). In the second study, 10 HEMOMAR-assisted sickle individuals with at least one tooth with a healthy crown without pulp calcification, clinical APN diagnosis using

PO and the cold pulp sensitivity test (n=27 teeth) were selected. Pulp calcifications, periodontal ligament and periodontal ligament were identified in tomographic analyzes. A bacterial detection was performed by means of bacterial culture, fluorescence and Polymerase Chain Reaction (PCR) in real time. The difference in prevalence of APN according to how co-variables color, sex, age and maxillaries were identified by Fisher's exact test ($\alpha=0.05$).

Results: In the first study, we observed 125 without APN and 15 with APN. As CoM were associated with the presence of APN (SFC=1.061, p=0.028). It was not explained by the No. SCCs/year (SFC = 0.1228, p = 0.579), nor by the body %SaO2 (SFC=0.1288, p=0.579). In the second study, the clinical diagnosis of APN was confirmed in most teeth (81.5%). NPA was more prevalent in the upper jaws (p=0.016). An amount of bacterial DNA detected was less than 100 copies/µL in only 18.5% of the sample. The imaging findings identified in 18.5% of the teeth were: thickening of the periodontal ligament 0.5 mm (n=3), hypercementose (n=1) and pulp node (n=1).

Conclusion: It was concluded that the presence of CoM in SCA individuals, mainly as osteoarticular lesions, increases the prevalence of APN in healthy teeth (first study). There is no need for immediate endodontic treatment in these teeth, but it is recommended periodic clinical and photographic proservation as a protocol to prevent infection in these individuals (second study).

Keywords: Sickle Cell Anemia. Dental Pulp Necrosis. Pulse Oximetry. Microbiology.

SUMÁRIO

1. INTRODUÇÃO

A Anemia Falciforme (AF) ocorre devido a uma mutação de ponto no gene da globina beta da hemoglobina, originando no lugar da hemoglobina normal (Hemoglobina A – HbA), uma hemoglobina alterada, denominada Hemoglobina S (HbS). Em situações persistentes de hipoxemia, as HbS podem sofrer polimerização, e consequente falcização das hemácias (assumindo forma de foice, daí o nome falciforme). Esses glóbulos vermelhos falciformes são mais aderentes ao endotélio vascular, possuem menor tempo de vida média, de 120 para 15 dias, e por isso se acumulam com mais facilidade nos vasos, gerando vasoclusão local, o que pode resultar em episódios de dor e lesão de órgãos vitais (OUIES; CASAMASSIMO; DA FONSECA, 2007; HEBBEL; VERCELLOTTI; NATH, 2009).

A AF é uma hemoglobinopatia transmitida como herança autossômica recessiva não ligada ao sexo (OUIES; CASAMASSIMO; DA FONSECA, 2007), predominante entre os afro-descendentes (WHO, 2011). Essa denominação é reservada para a forma da doença em homozigose, ou seja, quando a criança recebe de cada um dos pais um gene para HbS. Quando recebe de um dos pais um gene para HbS e outro para HbA, ela não tem a doença, é apenas portadora do Traço Falciforme (TF) (GILLIS; WEST, 2004).

A cada ano nascem no mundo cerca de 300 mil crianças portadoras de hemoglobinopatias, das quais mais de 200 mil são africanas com AF (WHO, 2011). No Brasil, nascem anualmente cerca de 3.500 crianças com AF e 200.000 portadoras de TF. A maior frequência é observada nas regiões Sudeste e Nordeste. A Bahia é o estado brasileiro com maior incidência de AF. Neste estado, entre os nascidos vivos, a incidência de AF é de 1:650 e de TF é de 1:17 (BRASIL, 2007). O Maranhão é o quarto estado brasileiro com maior índice desta doença, a cada 1.400 crianças nascidas vivas neste estado, uma possui AF e a cada 23 crianças uma possui TF (BATISTA; ANDRADE, 2008).

A AF tem sido apontada como uma questão de saúde pública (FRY, 2005), uma vez que acomete expressiva parcela da população mundial e brasileira (BRASIL, 2007; WHO, 2011), está associada à alta morbidade e mortalidade na infância, principalmente por sepse bacteriana, crise de sequestração esplênica, além da síndrome torácica aguda. A mortalidade das crianças abaixo dos cinco anos de idade chega a 25% dos casos (THOMAZ; PATTISON; SERJEANT, 1982).

As manifestações clínicas mais típicas desta doença são a anemia hemolítica crônica, oclusão da microvasculatura com isquemia tecidual, as crises dolorosas agudas osteoarticulares ou abdominais e lesões orgânicas crônica com asplenia funcional, vasculopatia cerebral, insuficiência renal, pulmonar e cardíaca. A variabilidade clínica da AF é resultado da combinação de fatores hereditários [mutação determinante da doença, níveis de Hemoglobina Fetal (HbF) e as interações com a talassemia alfa] e ambientais (renda familiar, tipo de alimentação, condições de saneamento básico e assistência médica) (PANTE-DE-SOUZA et al., 1998; GALACTEROS, 2001). Já a gravidade da AF é definida pela frequência de Crises Falcêmicas (CFs) (NAOUM et al., 2000; DE JESUS; ESCOBAR, 2002).

As CFs podem manifestar-se sob três formas: vasoclusiva, hemolítica ou aplástica. As crises *vasoclusivas* acontecem quando uma quantidade determinada de HbS polimeriza, ocorrendo o afoiçamento (falcização) das hemácias, que se tornam rígidas, aderem-se às paredes dos vasos sanguíneos, obstruindo-os e dificultando a circulação do sangue, principalmente nos capilares de fino calibre (HEBBEL, 1991; EMBURY, 2004). Na forma *hemolítica* são caracterizadas por uma anemia intensa decorrente da hemólise. Já as crises *aplásticas* estão associadas às infecções devido à ineficiência da medula óssea na produção de hemácias, causada por sua hiperatividade, ocasionando um quadro anêmico grave acompanhado de reticulopenia (NAOUM et al., 2000; de JESUS; ESCOBAR, 2002).

A partir das lesões de órgãos vitais decorrentes das CFs, sejam elas vasoclusiva, hemolítica ou aplástica, os indivíduos com AF podem desenvolver co-morbidades (CoM) agudas ou crônicas, elevando o índice de letalidade da doença. As mais comuns são as lesões osteoarticulares, alterações hepáticas e do sistema cardiovascular, e o Diabetes Mellitus Tipo-2 (DM-2) (FELIX; SOUZA; RIBEIRO, 2010). As lesões osteoarticulares são complicações da forma grave da AF, destacando-se a dactilite (síndrome mão-pé), caracterizada por um edema doloroso que pode acometer até as quatro extremidades do corpo; assim como a necrose isquêmica da cabeça do fêmur seguida de osteomelite, principalmente por *Salmonella* (ALMEIDA; ROBERTS, 2005). O aumento acentuado do débito cardíaco nestes indivíduos, decorrente da anemia hemolítica e hipertensão arterial pulmonar, induz a alterações cardiovasculares (FELIX; SOUZA; RIBEIRO, 2010; GLADWIN, 2016).

Indivíduos com AF também podem apresentar DM-2 e alterações hepáticas como resposta secundária às lesões no pâncreas e fígado, devido ao acúmulo excessivo de ferro (hemocromatose) nas células beta-pancreáticas e hepáticas, consequência da destruição precoce das hemácias falcizadas seguida de múltiplas transfusões sanguíneas (CANÇADO; JESUS, 2007; FELIX; SOUZA; RIBEIRO, 2010).

Não há tratamentos para impedir a falcização das hemácias. Entretanto, para amenizar as consequências da AF, o tratamento consiste na inclusão de medidas preventivas para as CFs. Estas medidas incluem boa nutrição; profilaxia, diagnóstico e terapêutica precoce de infecções; manutenção de boa hidratação e evitar condições climáticas adversas. Aliados a tais medidas preventivas, a prescrição do ácido fólico aos pacientes com AF é indispensável para maturação e velocidade na produção dos glóbulos vermelhos (GALACTEROS, 2001; DE MONTALEMBERT, 2008).

Atualmente, o protocolo de tratamento dos pacientes falcêmicos também inclui o uso da hidroxiuréia (HU), muitas vezes como a medicação de primeira escolha. Esse quimioterápico aumenta os níveis de HbF, melhora a gravidade clínica da doença e os parâmetros hematológicos, reduz a frequência e intensidade das crises álgicas, a duração das internações, o número de transfusões, além de diminuir em 50% a ocorrência de novos episódios de síndrome torácica aguda, principal causa de morte em indivíduos com AF (LANZKRONET et al., 2008).

As manifestações patológicas desta doença já demonstradas em tecidos mineralizados e conectivos em outras áreas do corpo, tais como rins, fígado, pulmões e coração, também ocorrem em tecidos bucais. Entretanto, não são sinais patognomônico da AF (BOTELHO et al., 2009). Na cavidade bucal podem ocorrer isquemia e necrose dos ossos faciais, o que pode desencadear osteomielite, principalmente na mandíbula, sem causa dental (ALMEIDA; ROBERTS, 2005; AL-JAFFAR et al., 2016). Já foi demonstrado que a vasoclusão da microcirculação da polpa dental em dentes hígidos pode resultar em pulpite (PLATT et al., 1991; VGONTZAS; CHALESTON; ROBBINS, 2006) e até Necrose Pulpar Assintomática (NPA) (ANDREWS; ENGLAND; KEMP, 1983; BISHOP; BRIGGS; KELLEHER, 1995; DEMIRBAS; AKTENER; ÜNSAL, 2004; COSTA; THOMAZ; SOUZA, 2013).

Estudos prévios identificaram a NPA em dentes hígidos como a principal manifestação bucal desta doença (ANDREWS; ENGLAND; KEMP, 1983; BISHOP; BRIGGS; KELLEHER, 1995). Demirbas, Aktener e Ünsal (2004) alertaram que a AF pode causar NPA sem necessariamente ter uma etiologia associada à cárie ou às doenças periodontais. Costa, Thomaz e Souza (2013), empregando análise multivariada em modelos estatísticos ajustados para possíveis covariáveis de confundimento, estimaram que a ocorrência de NPA em dentes higídos é 8.33 vezes maior em indivíduos com AF do que naqueles sem a doença. Esses autores, mesmo na ausência de evidências científicas anteriores, sugerem que o órgão pulpar de dentes com coroas hígidas de indivíduos com AF seja passível de ser comprometido por microtrombos de células falciformes durante as CFs, causando bloqueio dos vasos que o irrigam, levando-o à necrose por coagulação, asséptica ou não, uma vez que bactérias oriundas das recorrentes infecções de outros órgãos vitais poderiam alojar-se na polpa dental via anacorese. Portanto, parece-nos plausível que dentes hígidos com NPA em indivíduos com AF possuam uma microbiota endodôntica.

Considerando o exposto, o presente estudo foi dividido em dois capítulos: Capítulo I – Mecanismos biológicos associados a necrose pulpar assintomática de individuos com anemia falciforme: um estudo de coorte prospectivo e Capítulo II – Há necessidade de tratar dentes hígidos com necrose pulpar assintomática de indivíduos com anemia falciforme? O primeiro teve como objetivo explicar os mecanismos envolvidos na associação entre a AF e NPA e o segundo, investigar a necessidade de tratamento endodôntico desses dentes por meio da avaliação das características clínicas e macroscópicas do órgão pulpar, dos aspectos imagenológicos da câmara pulpar e região periapical, e da presença de microrganismos no sistema de canais radiculares.

2. CAPÍTULO I

MECANISMOS BIOLÓGICOS ASSOCIADOS A NECROSE PULPAR ASSINTOMÁTICA DE INDIVIDUOS COM ANEMIA FALCIFORME: UM ESTUDO DE COORTE PROSPECTIVO

(a ser submetido ao *Journal of Dental Research*)

Resumo

O objetivo deste estudo de coorte foi investigar quais os mecanismos biológicos envolvidos na associação entre Necrose Pulpar Assintomática (NPA) e Anemia Falciforme (AF), após seis anos de prospecção. Esse estudo teve duas fases: uma retrospectiva e outra prospectiva. Na fase retrospectiva (2010-2011) foram selecionados 113 indivíduos com AF maiores de 16 anos, com pelo menos um dente hígido, sem histórico de parestesia do nervo alveolar inferior nos últimos seis meses, traumatismos dentários ou doença periodontal. Na fase prospectiva (2015-2016), sete indivíduos morreram e 25 não foram encontrados. Foram incluídos 59 novos participantes, totalizando uma amostra de 140 indivíduos, 125 sem NPA e 15 com NPA. Um modelo teórico foi construído para explorar três mecanismos biológicos envolvidos na associação NPA-AF: o aumento do Número de Crises Falcêmicas no último Ano (Nº CFs/ano); menor porcentagem de Saturação Arterial de Oxigênio (%SaO_2) corpórea e a presença de Comorbidades (CoM). Nº CFs/ano e %SaO_2 corpórea foram consideradas variáveis mediadoras, e CoM (cardiopatias, alterações hepáticas, diabetes mellitus tipo-2 e lesões osteoarticulares), a variável latente explicativa. O modelo teórico proposto para testar a causalidade da associação foi analisado por Modelagem com Equações Estruturais. As prevalências das variáveis entre os grupos com e sem NPA foram comparadas pelos testes t de Student ou exato de Fisher. O nível de significância adotado para todas as análises foi de 5%. A NPA foi associada à presença de CoM (CPF=1.115; P=0.032). Entretanto, essa associação não foi mediada pelo Nº CFs/ano (CPF=0.127; P=0.596), nem pela %SaO_2 corpórea (CPF= -0.102; P=0.485). As prevalências de lesões osteoarticulares (P=0.009) e óbitos (P<0.001) foram maiores em indivíduos com NPA. As CoM, principalmente lesões osteoarticulares, aumentam a prevalência de NPA em dentes hígidos de falcêmicos. A NPA pode ser considerada um indicador de letalidade da AF.

Palavras-chave: anemia falciforme, polpa dentária, necrose pulpar dental, oximetria de pulso, comorbidades

INTRODUÇÃO

A anemia falciforme é a doença genética mais prevalente em todo mundo, manifestando-se em homozigose (HbSS). Caracteriza-se por ser uma anemia hemolítica com a presença da Hemoglobina S (HbS) dentro das hemácias (Adeyemo et al. 2011) e, consequentemente, hipóxia tecidual, uma vez que HbS é menos efetiva no transporte de oxigênio que a hemoglobina normal (Naoum 2000).

Quando uma determinada quantidade de HbS polimeriza, ocorre a falcização das hemácias, que se tornam rígidas e mais aderentes às paredes dos vasos sanguíneos, dificultando a circulação do sangue, especialmente nos capilares de fino calibre resultando em vasoclusão (Hebbel 1991; Embury 2004).

A vasoclusão é uma das três formas de crises falcêmicas (Hebbel 1991; Embury 2004).A frequência dessas crises durante o ano determina a gravidade clínica da anemia falciforme, podendo resultar em lesões de órgãos vitais (Naoum 2000; de Jesus; Escobar 2002), e possivelmente seria uma explicação para o comprometimento da vitalidade da polpa dentária de dentes hígidos (Dermibas Kaya et al. 2004; Costa et al. 2013). As crises falcêmicas também podem ocorrer na forma hemolítica, anemia intensa decorrente da hemólise, ou ainda na forma aplástica, associada às infecções devido à ineficiência da medula óssea na produção de hemácias (Naoum 2000; de Jesus; Escobar 2002).

A partir das lesões de órgãos vitais decorrentes das crises falcêmicas vasoclusivas, hemolíticas ou aplásticas, os indivíduos HbSS podem desenvolver comorbidades, sendo às mais comuns as lesões osteoarticulares, alterações hepáticas, alterações do sistema cardiovascular e o Diabetes Mellitus Tipo-2 (DM-2) (Felix et al. 2010), elevando o índice de letalidade da doença.

Há evidências da associação entre anemia falciforme e necrose pulpar assintomática em dentes hígidos (Costa et al. 2013), entretanto ainda se desconhece os mecanismos biológicos envolvidos nessa associação. Assim, assumimos como hipótese que o aumento do Número de Crises Falcêmicas no último Ano (Nº CFs/ano), menor saturação Arterial de Oxigênio (%SaO_2) corpórea e presença de Comorbidades (CoM) nos indivíduos HbSS resultariam em necrose pulpar assintomática. Portanto, o objetivo deste estudo foi analisar o efeito do Nº CFs/ano, da %SaO_2 corpórea e da presença de CoM na necrose pulpar assintomática em dentes hígidos de indivíduos HbSS.

MÉTODOS

Trata-se de um estudo de coorte. Esse estudo teve duas fases: uma retrospectiva e outra prospectiva. A fase retrospectiva foi realizada entre junho de 2010 e junho de 2011 na Supervisão de Hematologia e Hemoterapia do Maranhão (HEMOMAR), São Luís, MA, Brasil, após aprovação do Comitê de Ética em Pesquisa (CEP) local sob protocolo n° 23115 004993/ 2010-71. Os participantes do estudo foram selecionados aleatoriamente pela condição de exposição (Anemia Falciforme – AF), diagnosticada por meio da eletroforese da hemoglobina ao nascimento. Essa informação foi obtida nos prontuários eletrônicos da HEMOMAR. Os critérios de elegibilidade foram ser maior de 16 anos e ter pelo menos um dente com coroa hígida, sem histórico de parestesia do nervo alveolar inferior nos últimos seis meses, sem história prévia de traumatismo dentário e sem doença periodontal. O diagnóstico do desfecho (Necrose Pulpar Assintomática – NPA) foi obtido por meio da Oximetria de Pulso adaptada à Odontologia (OP) e do Teste de Sensibilidade Pulpar (TSP) ao frio (Costa et al. 2013).

A fase prospectiva (primeiro seguimento) ocorreu no período de setembro de 2015 a setembro de 2016 (protocolo CEP n° 57953814.9.0000.5087). Os participantes do estudo (n=113) foram convidados a comparecer à HEMOMAR. Os procedimentos para obtenção do diagnóstico da condição pulpar foram repetidos em todos os dentes com coroas hígidas, de forma idêntica a primeira fase.

Estimou-se que uma amostra de 140 indivíduos HbSS (125 sem NPA e 15 com NPA) teria o poder de 99.8% para identificar diferenças entre as médias de %SaO_2 pulpar nos grupos do estudo, considerando alpha de 5%. Admitiu-se um número médio de 12.55 (±3.6) de N° CFs/ano para indivíduos HbSS com NPA, e sem NPA de 13.5 (±2.9), de acordo com dados preliminares do estudo.

No seguimento, os participantes da primeira fase do estudo (n=113) foram novamente confirmados pelos critérios de elegibilidade. Dos 113 indivíduos (11 com NPA e 102 sem NPA), 32 não retornaram para reavaliação (oito com NPA e 24 sem NPA). Desses, quatro com NPA e três sem NPA morreram. Os demais (n=25), não foram encontrados. Para completar o tamanho da amostra, novos participantes (n=59) foram selecionados por meio de sorteio casual simples, a partir do cadastro da Assistência Social da HEMOMAR (Fig. 1) e foram submetidos aos mesmos critérios de elegibilidade.

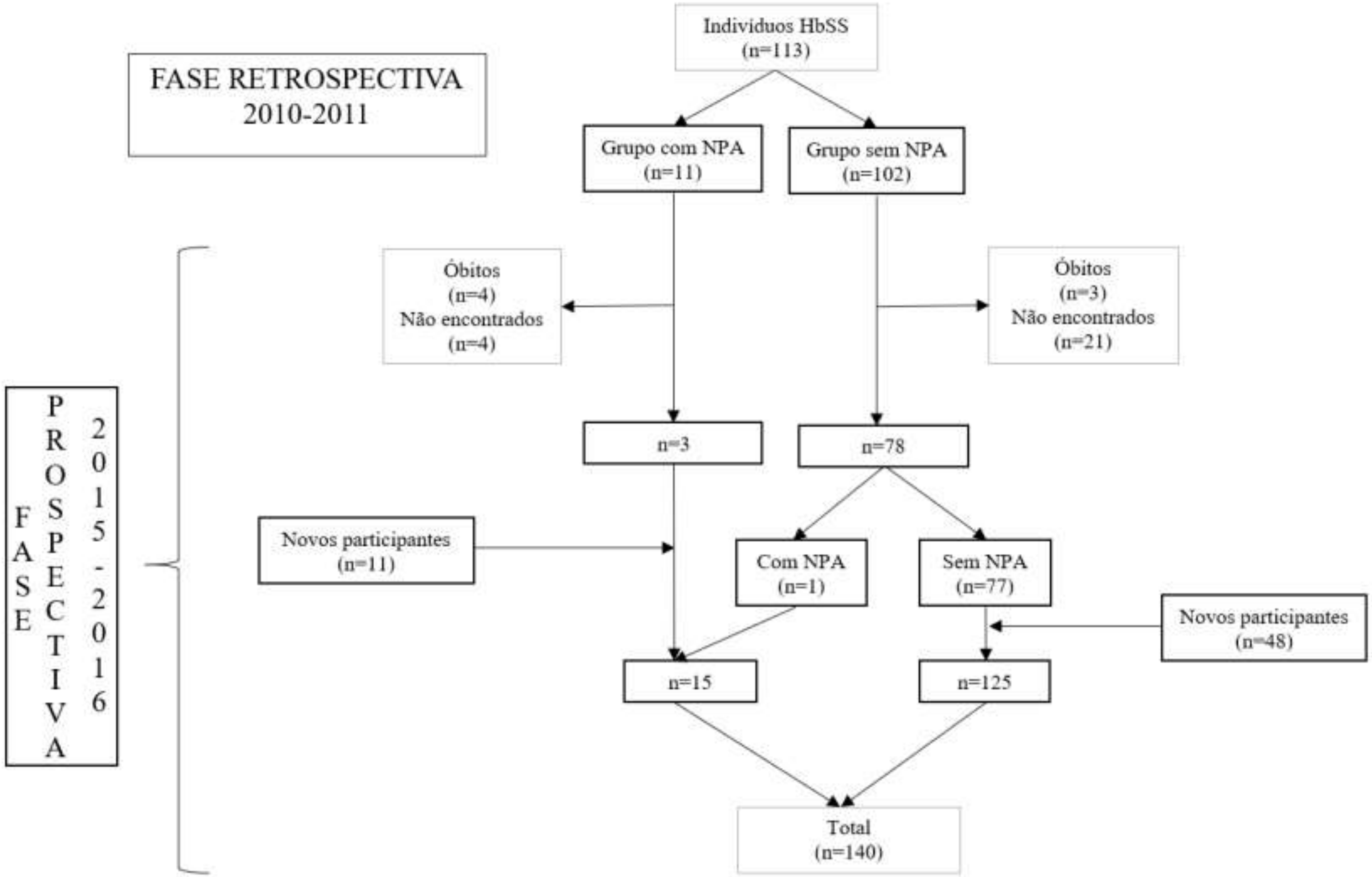

Figura 1. Fluxograma do estudo e da seleção dos novos participantes da coorte. NPA, Necrose Pulpar Assintomática.

As variáveis Nº CFs/ano (número de crises falcêmicas do último ano), presença de cardiopatias (sim ou não), alterações hepáticas (sim ou não), DM-2 (sim ou não), lesões osteoarticulares (sim ou não), cor auto-referida (branca, parda ou preta), sexo (masculino ou feminino), idade (em anos), Classe Socio-Econômica (CSE), segundo a Associação Brasileira de Estudos e Pesquisas (A/B; C ou D/E) (ABEP 2015) e uso de Hidroxiúreia – HU (sim ou não) foram coletadas por meio de entrevista, empregando um questionário estruturado.

A hidroxiúreia é um fármaco estimulador da síntese de cadeia globínica gama que aumenta a síntese intraeritrocitária de Hemoglobina Fetal (HbF). Altos níveis dessa hemoglobina previne várias complicações da anemia falciforme, que a longo prazo tem impacto positivo na qualidade de sobrevida desses pacientes, com redução no número de hospitalizações, tempo de internação, menor ocorrência de síndrome torácica aguda e menor necessidade de transfusão eritrocitária (Cançado; Jesus 2007).

O diagnóstico da NPA foi obtido da forma já descrita anteriormente (Costa et al. 2013). As mensurações da %SaO_2 corpórea e pulpar foram realizadas com o auxílio do Oximetro de Pulso MD300A (IMFtec Tecnologia para Saúde LTDA, São Paulo, SP, Brasil). A %SaO_2 corpórea foi mensurada no dedo indicador direito e a pulpar, no centro das faces vestibular e palatal/lingual dos dentes. O sensor do oxímetro possui dois diodos, um receptor e outro emissor; os quais foram mantidos paralelos entre si durante as mensurações obtidas através do dente ou dedo. As mensurações foram realizadas em triplicatas. Utilizou-se a mediana dos três valores (Costa et al. 2013).

Para não influenciar a leitura do oxímetro no momento da mensuração da %SaO_2 pulpar a luz do refletor foi desligada e o indivíduo foi orientado a manter a cabeça imóvel. Os dentes foram considerados com NPA quando apresentaram mediana da %SaO_2 pulpar $\leq$ 79% (Calil et al. 2008).

A resposta ao TSP ao frio foi registrada como positiva ou negativa. Para isso, o gás diclorodifluormetano (-50°C; Endofrost, Roeko, Langenau, Alemanha) foi aplicado no centro do terço cervical da face vestibular do dente selecionado com ajuda de um microbrush. Cada paciente foi orientado a levantar o antebraço e a mão esquerda imediatamente após apresentar sensibilidade (resposta positiva). A NPA foi constatada quando o paciente não apresentava sensibilidade ao teste (resposta negativa).

Um modelo teórico foi construído para explorar três possíveis mecanismos biológicos explicativos principais para a NPA em indivíduos HbSS: 1) o aumento do Nº CFs/ano; 2) a menor %SaO_2 corpórea e 3) a presença de CoM. O mecanismo CoM foi formado por quatro indicadores: presença de cardiopatias, alterações hepáticas, DM-2 e lesões osteoarticulares (Fig. 2).

As demais variáveis observadas foram cor auto-referida, sexo, idade, CSE, uso de HU e taxa de sobrevivência (número de óbitos durante os seis anos de seguimento dessa coorte).

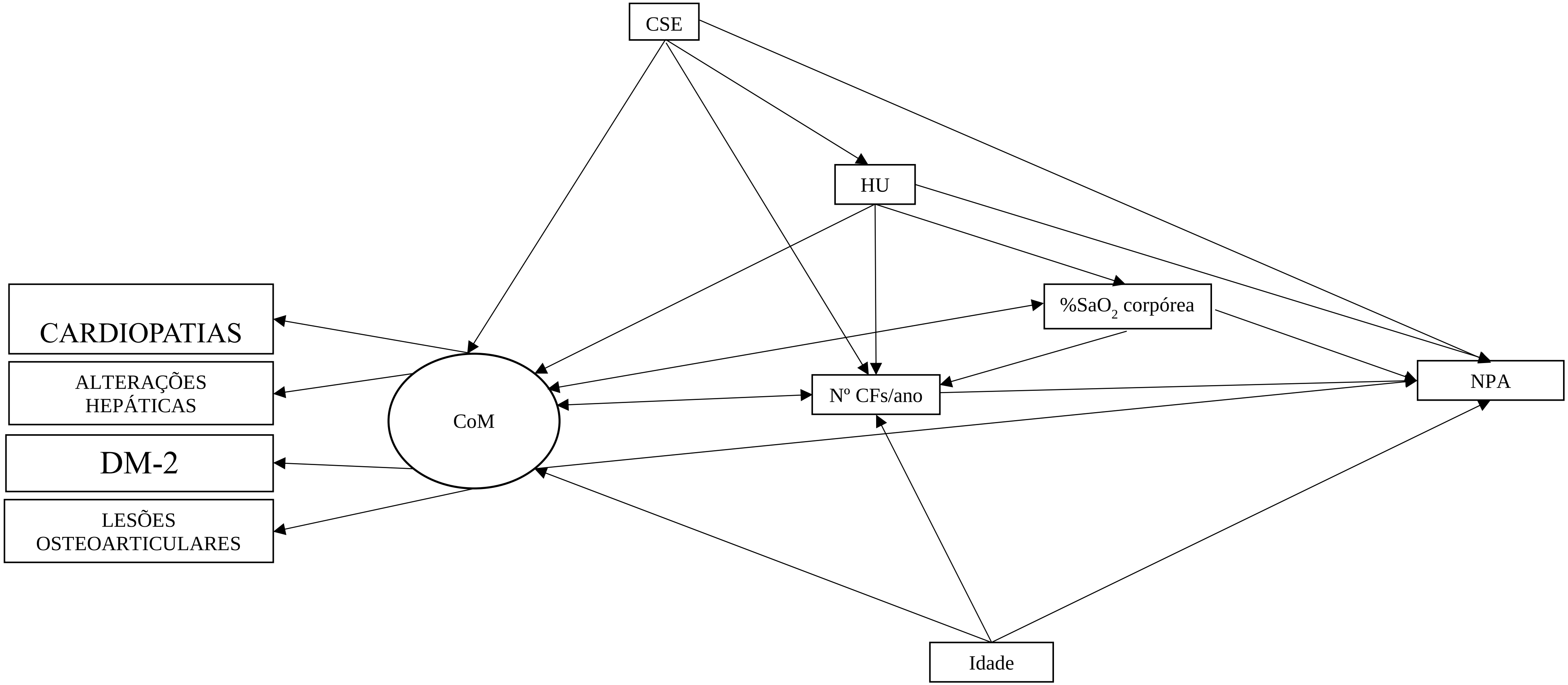

Figura 2. Modelo teórico do estudo (desfecho=ter NPA). NPA, Necrose Pulpar Assintomática. DM-2, Diabetes Mellitus Tipo-2. CoM, Comorbidades (variável latente). CSE, Classe Sócio-Econômica. HU, Hidroxiúreia. N° CFs/ano, Número de Crises Falcêmicas no último Ano. $\%SaO_2$, Porcentagem de Saturação Arterial de Oxigênio.

Análise estatística

A normalidade da distribuição dos dados foi testada por análise gráfica e teste de Kolmogorov-Smirnov. As prevalências entre as variáveis do estudo foram comparadas entre os grupos com e sem NPA por meio dos testes t de Student ou exato de Fisher.

No modelo teórico testado para explicar a associação entre NPA-AF os dois mecanismos, aumento do Nº CFs/ano e menor %SaO_2 corpórea, foram considerados variáveis mediadoras dessa associação e a CoM, a variável latente. Idade, classe socioeconômica e uso de HU foram consideradas variáveis confundidoras (Fig. 2). A taxa de sobrevivência não foi incluída no modelo teórico do estudo por que é um dado referente apenas a fase prospectiva da coorte.

O modelo teórico proposto foi analisado por meio da Modelagem com Equações Estruturais (MEE). O nível de significância adotado para todas as análises foi de 5%. As análises foram realizadas com o software STATA/SE 12.0 (Statacorp LLC, College Station, Texas, EUA) e Mplus versão 7.0 (Muthén & Muthén, Los Angeles, CA, EUA).

A MEE consiste em dois sub-modelos: o modelo de mensuração, que estabelece como o constructo (variável latente) é medido; e o modelo estrutural, que analisa o modelo teórico como um todo, na análise de caminhos, onde as associações entre as variáveis são determinadas. A variável latente é representada por círculo ou elipse e as variáveis observadas são representadas por quadrados ou retângulos (Kline 2011).

Uma boa variável latente apresenta validade convergente, mostrando que os seus indicadores medem o mesmo constructo, verificado pelas cargas fatoriais com valores superiores a 0.30 (Tabachnick; Fidell 2007). Além disso, deve haver validade discriminante, ou seja, as correlações entre indicadores não deve ser excessivamente alta (>0.90), demonstrando que cada indicador mede aspectos distintos do constructo (Kline 2011).

As Cargas Fatoriais Padronizadas (CFP) do modelo estrutural foram interpretadas de acordo com Kline (2011), onde coeficientes com valores próximos a 0.10 indicam um efeito pequeno, 0.30 efeito médio e superior a 0.50, efeito forte.

Para a escolha do modelo final, foram analisados os seguintes índices de qualidade de ajuste dos modelos: teste do Qui-quadrado (χ^2); erro médio quadrático de aproximação (Root Mean Square Error of Approximation–RMSEA); limite superior do intervalo de confiança de 90% do RMSEA; índices incrementais Comparative Fit Index (CFI) e Tucker-Lewis Index (TLI); e Weighted Root Mean Square Residual (WRMR). Os modelos foram considerados bem ajustados quando o valor do χ^2 foi maior que 0.05; RMSEA menor que 0.5; limite superior do IC90% do RMSEA menor que 0.08; CFI e TLI maiores que 0.95; e WRMR menor que 1.0 (Farias; Santos 2000; Cook et al. 2009; Léon 2011).

RESULTADOS

As prevalências de lesões osteoarticulares e óbitos foram maiores em indivíduos HbSS com NPA (*P*=0.009 e *P*<0.001, respectivamente). Para as demais variáveis, não houve diferença entre os grupos (Tab. 1).

Tabela 1. Caracterização da amostra.

Variáveis	NPA (n=140)				*P* – valor
	Não (n= 125)		Sim (n=15)		
	n	%	n	%	
CSE					
B	27	21.6	5	33.3	0.197[1]
C	78	61.4	10	66.7	
D	20	16	0	0	
Cor					
Preta	74	59.2	11	73.3	0.507[1]
Parda	47	37.6	4	26.7	
Branca	4	3.2	0	0	
DM-2					
Não	125	100	14	93.3	0.107[1]
Sim	0	0	1	6.7	
Cardiopatias					
Não	116	92.8	14	93.3	0.709[1]
Sim	9	7.2	1	6.7	
Alterações hepáticas					
Não	114	91.2	15	100	0.608[1]
Sim	1	8.8	0	0	
Lesões osteoarticulares					
Não	123	98.4	12	79.9	0.009[1]*
Sim	2	1.6	3	20.1	
Uso de HU					
Não	18	14.4	2	13.3	0.636[1]
Sim	107	85.6	13	86.7	
Taxa de sobrevivência†					
Óbitos	3	3	4⋆	36.3⋆	<0.001[1]*
Vivos	99	97	7	63.7	
	X	**Dp**	**X**	**Dp**	***P* – valor**
Idade	31.93	9.5	26.1	11.9	0.280[2]
Nº CFs/ano	12.55	3.6	13.5	2.9	0.107[2]
%SaO_2 corpórea	86.65	4.0	84.9	2.9	0.351[2]

Abreviação: NPA, Necrose Pulpar Assintomática. CSE, Classe Sócio-Econômica. DM-2, Diabetes Mellitus Tipo-2. HU, Hidroxiúreia. Nº CFs/ano, Número de Crises Falcêmicas no último Ano. %SaO_2, Porcentagem de Saturação de Arterial de Oxigênio. n, frequência absoluta. %, frequência relativa. x, média. Dp, desvio padrão.

[1]Exato de Fisher.

[2]t de Student.

*Estatisticamente significante.

†Porcentagem calculada para o tamanho amostral da fase retrospectiva da coorte (n=113): grupo sem NPA (n=102) e grupo com NPA (n=11).

⋆Após a conclusão do primeiro seguimento (2017) morreu mais um participante do grupo com NPA, não incluído na análise (n=5; 45.5%).

O modelo testado apresentou bons índices de ajuste: o teste χ^2 comparou o modelo hipotético ideal com o modelo proposto e não apresentou diferenças significantes (P>0.05). O valor do RMSEA foi menor que o máximo aceitável (<1.0) e o limite superior do IC90% foi menor que 0.08, demonstrando semelhança entre o modelo examinado e o modelo hipotético. No modelo, os índices CFI e TLI são superiores a 0.95 e o WRMR é inferior a 0.95 (Tab. 2).

Tabela 2. Índices de ajuste do modelo teórico do estudo.

Índices de ajuste	**Modelo (Desfecho: ter NPA)**
	Valores
Nº de parâmetros livres	31
Graus de liberdade	23
χ^{2a}	21.8
P-valor do χ^2	0.532
RMSEA[b]	<0.001
IC90% do RMSEA [c]	0.000-0.065
CFI[d]	1.00
TLI[e]	1.06
WRMR[f]	0.64

Abreviação: %, porcentagem.
[a]TesteQui-Quadrado–χ^2 (referência: menor valor).
[b]Root Mean Square Error of Approximation–RMSEA (referência: menor que .05).
[c]Intervalo de Confiança a 90% (referência: limite superior do IC90% menor que 0.08).
[d]Comparative Fit Index–CFI (referência: maior que 0.95).
[e]Tucker Lewis Index–TFI (referência: maior que 0.95).
[f]Weighted Root Mean Square Residual–WRMR (referência: menor que 1.0)

No modelo testado, todas as CFP que formaram o constructo CoM foram maiores que 0.30 e estatisticamente significantes (P<0.05; Fig. 3), indicando boa validade convergente. Porém, uma das variáveis observadas (DM-2) apresentou CFP maior que 0.95, indicando que sozinha explica grande parte da variabilidade do construto (Fig. 3).

Foi observada associação negativa entre HU e CoM (CFP= -0.315; P=0.014). Houve associação positiva da idade do paciente com CoM (CFP=0.680; P<0.001; Tab. 3) e da CoM com a presença de NPA (CFP=1.115; P=0.032). As variáveis consideradas no modelo explicaram 64.8% da variabilidade da NPA (R^2=0.648; P=0.009; Tab. 3).

CSE, Idade e HU são responsáveis por 56.5% da variabilidade da CoM (R^2=0.565; P=0.015; Tab. 3).

Tabela 3. Efeitos diretos das variáveis do estudo nas análises de equações estruturais.

Variáveis	Modelo (desfecho: ter NPA)		
	CFP	***P* – valor**	**R^2**
CoM			0.565*
CSE	-0.083	0.747	
Idade	0.680*	<0.001*	
HU	-0.315*	0.014*	
Nº CFs/ano			0.005
CoM	-0.200	0.935	
CSE	0.007	0.733	
Idade	-0.011	0.895	
HU	0.051	0.500	
%SaO_2 corpórea	-0.054	0.453	
HU			0.008
CSE	0.090	0.554	
%SaO_2 corpórea			0.006
CoM	0.165	0.299	
HU	0.080	0.546	
NPA			0.648*
CoM	1.115*	0.032*	
CSE	-0.124	0.683	
Idade	-1.058	0.061	
HU	0.363	0.159	
Nº CFs/ano	0.127	0.596	
%SaO_2 corpórea	-.0.102	0.485	

Abreviação: NPA, Necrose Pulpar Assintomática. CoM, Comorbidades. CSE, Classe Sócio-Econômica. HU, Hidroxiúreia. Nº CFs/ano, Número de Crises Falcêmicas no último Ano. %SaO_2, Porcentagem de Saturação Arterial de Oxigênio. CFP, Cargas Fatoriais Padronizadas. R^2, coeficiente de associação.
*Estatisticamente significante.

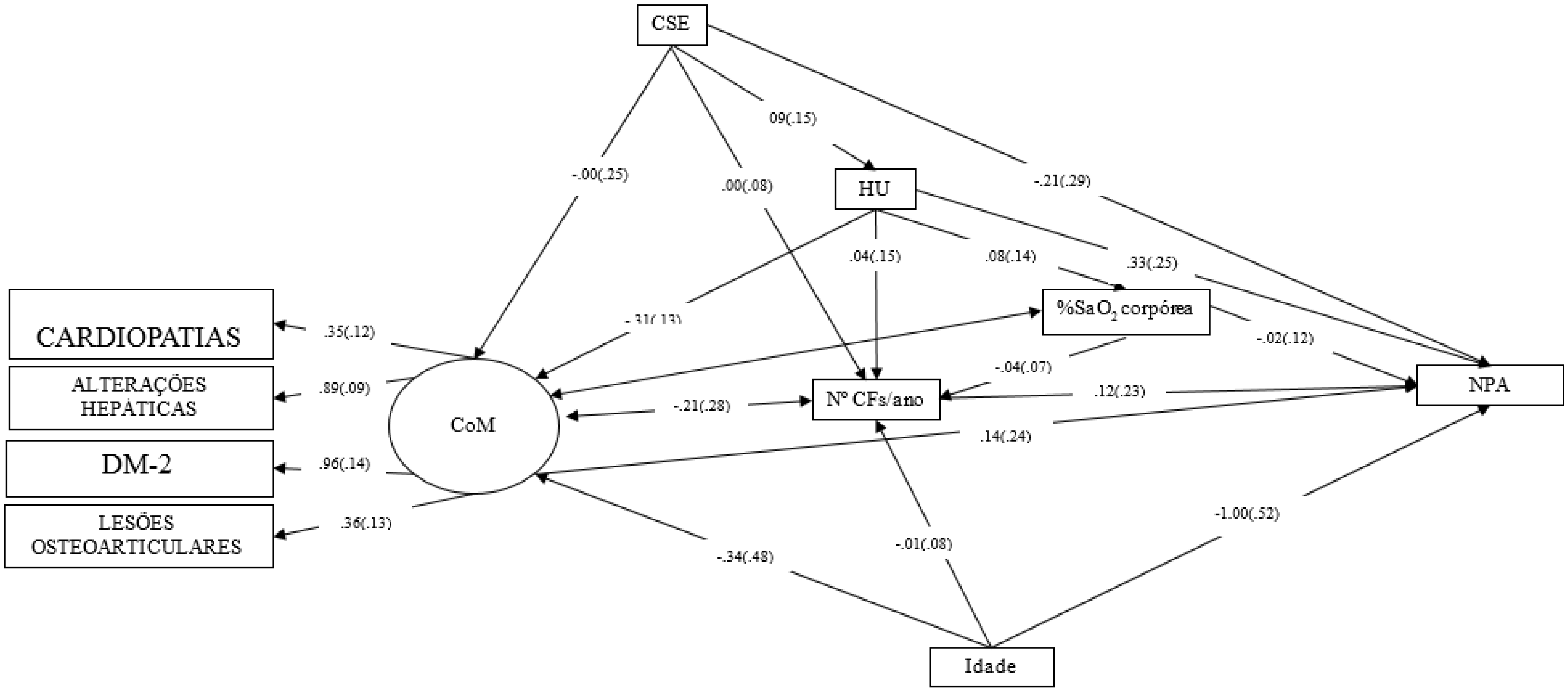

Figura 3. Modelo testado por meio da Modelagem de Equações Estruturais (desfecho=ter NPA). NPA, Necrose Pulpar Assintomática. DM-2, diabetes mellitus tipo-2. CoM, Comorbidades (variável latente). CSE, Classe Sócio-Econômica. HU, Hidroxiúreia. N° CFs/ano, Número de Crises Falcêmicas no último Ano. %$SaO_{2,}$ Porcentagem de Saturação Arterial de Oxigênio.

Tabela 4. Efeitos totais e indiretos da CSE, HU e Idade sobre NPA.

Variáveis	**Modelo**	
	CFP	***P* – valor**
Efeito de CSE para NPA		
Total	-0.214	0.119
Indireto total	-0.091	0.746
Indiretos específicos		
CSE→HU→NPA	0.033	0.590
CSE→Nº CFs/ ano→NPA	0.001	0.937
CSE→CoM→NPA	-0.092	0.743
CSE→HU→%SaO_2→NPA	-0.001	0.781
CSE→HU→CoM→NPA	-0.032	0.587
CSE→HU→%SaO_2→ Nº CFs/ano →NPA	0.000	0.760
Efeito de HU para NPA		
Total	0.010	0.952
Indireto total	-0.353	0.126
Indiretos específicos		
HU→%SaO_2 corpórea→NPA	-0.008	0.682
HU→Nº CFs/ano→NPA	0.007	0.755
HU→CoM→NPA	-0.351	0.136
HU→%SaO_2 corpórea → Nº CFs/ano →NPA	-0.000	0.728
Efeito de IDADE para NPA		
Total	-0.301*	0.004*
Indireto total	0.757	0.154
Indiretos específicos		
Idade→Nº CFs/ano→NPA	-0.001	0.897
Idade→CoM→NPA	0.758	0.155

Abreviação: Nº CFs/ano, Número de Crises Falcêmicas no último Ano. CoM, Comorbidades. CFP, Cargas Fatoriais Padronizadas. NPA, Necrose Pulpar Assintomática. %SaO_2, Porcentagem de Saturação Arterial de Oxigênio. CSE, Classe Sócio-Econômica. HU, Hidroxiúreia.
*Estatisticamente significante.

O Nº CFs/ano (CPF=0.127; *P*=0.596; Tab. 3) e a %SaO_2 corpórea (CPF= -0.102; *P*=0.485; Tab. 3) não foram associadas à presença de NPA no modelo testado. As CoM e a idade apresentaram efeito sobre a NPA. As CoM apresentaram efeito direto significante (CFP=1.115; *P*=0.032; Tab. 3), indicando que quanto maior a CFP das CoM maior a probabilidade da presença de NPA. Por outro lado, a idade do paciente apresentou efeito total significante sobre a ocorrência de NPA (CFP= -0.301; *P*=0.004; Tab. 4), indicando que quanto maior a idade do paciente, menor a probabilidade de apresentar NPA.

DISCUSSÃO

Estudos prévios sugerem que a NPA é consequência do acúmulo das hemácias falcizadas na microcirculação pulpar (Bishop et al. 1985; Andrews et al. 1986; Dermibas et al. 2004). Costa et al. (2013) estimaram que o indivíduo HbSS tem 8.3 mais risco de ter dentes hígidos com NPA, entretanto, não esclareceram a relação causal desta associação. Para isso, neste estudo de coorte foram testados três mecanismos biológicos: o aumento do Nº CFs/ano, a diminuição da %SaO_2 corpórea e a presença de CoM.

O primeiro mecanismo explorado para explicar a associação foi que o Nº CFs/ano estaria associado à NPA, supondo-se que episódios de vasoclusão freqüentes poderia resultar em perda da vitalidade pulpar, semelhante ao que acontece quando a circulação periférica destes indivíduos é obstruída provocando necrose tecidual (Naoum 2000; de Jesus; Escobar 2002). A plausibilidade biológica para tal hipótese sustenta-se no fato de que as CFs podem manifestar-se sob três formas: vasoclusiva, hemolítica ou aplástica. Entretanto, no presente estudo o Nº CFs/ano não explicou a NPA; o que poderia ser atribuído ao fato de que nem sempre as crises falcêmicas são vasoclusivas (Naoum 2000;

de Jesus; Escobar 2002) e, portanto, nem sempre haverá o risco de NPA durante esses eventos.

O segundo mecanismo testado foi a diminuição da %SaO_2 corpórea, que também não explicou a associação NPA-AF. A hipóxia corporal transitória em indivíduos HbSS não reduz de forma crítica a perfusão periferica, onde as demandas de Hb e, consequentemente de oxigênio e fluxo sanguíneo regional são menores (Coates, 2018). Ademais, esses parâmetros variam em diferentes partes do corpo e podem não ser adequadamente refletido por medições feitas na ponta do dedo (Coates, 2018), local onde foi estimada %SaO_2 corpórea.

O terceiro mecanismo analisado, as CoM, explicaram a associação com a necrose pulpar assintomática em indivíduo HbSS. As comorbidades agravam o estado pró-inflamatório constante em que o indivíduo HbSS vive desde a infância até a vida adulta. Esse quadro pró-inflamatório se manifesta como uma hemólise crônica grave com complicações vasoclusivas, vasculopatia e consequentes injúrias agudas e crônicas, dentre elas a necrose pulpar assintomática. Entretanto, esse quadro não está associado às crises falcêmicas decorrentes da hipóxia corporal devido a uma infecção, desidratação e/ou tensão emocional de qualquer natureza (Galiza-Neto Pitombeiras 2003; Felix et al. 2010).

Ter lesões osteoarticulares foi o único indicador da variável latente CoM que apresentou associação com a necrose pulpar assintomática (Tab. 1 e Fig. 4). Essa associação tem plausibilidade biológica pois estas duas condições podem ter os mesmos fatores etiológicos em indivíduo HbSS, podem ocorrer devido à falcização das hemácias, isquemia e infarto dos tecidos afetados, de fenômenos tromboembólicos ou diminuição do fluxo sanguíneo local (Almeida; Roberts 2005; Costa et al. 2013).

Nos nossos achados, foi observada associação entre necrose pulpar assintomática e maior taxa de óbitos dos participantes da coorte (Tab. 1). Este, foi o primeiro estudo de coorte que apontou a necrose pulpar assintomática no baseline como preditor de risco para as formas mais letais de danos orgânicos irreversíveis que podem acometer os indivíduos HbSS. Um estudo de coorte prospectivo anterior (1959-1999), já apontou alterações ósseas crônicas como marcadores de vasculopatia falciforme, associadas aos estágios finais das doenças renal, pulmonar crônica e acidente vascular encefálico (Powards et al. 2005).

No presente estudo as lesões osteoarticulares analisadas no seguimento da coorte foram associadas a ter dentes hígidos com necrose pulpar assintomática. Esse achado sugere que essas duas condições sejam indicadores de vasculopatia falciforme, com progressão para letalidade da anemia falciforme. Essa especulação ganha força com a confirmação de que após a conclusão do seguimento dessa coorte (2015-2016), mais um participante da amostra com necrose pulpar assintomática (não incluido na análise estatística) veio a óbito (Tab. 1).

O uso de hidroxiuréia foi associado com menores valores para comorbidades (Tab. 3). Esse achado pode ser explicado pelas estratégias de políticas públicas adotadas no Brasil para o tratamento das doenças falciformes (Lanzkron et al. 2008). O tratamento hematológico da anemia falciforme avançou bastante nas últimas décadas, e atualmente, o protocolo de tratamento destes pacientes inclui o uso da hidroxiureia como o fármaco de primeira escolha, que comprovadamente reduz as comorbidades e a taxa de mortalidade da doença (Lanzkron et al. 2008). Uma revisão sistemática sobre o uso da hidroxiuréia como tratamento para as doenças falciformes, mostra evidências de que sua ação aumenta os parâmetros hematológicos melhorando a gravidade clínica destas doenças como a frequência e intensidade das crises álgicas, diminuindo em até 50% a ocorrência de novos episódios de síndrome torácica aguda, principal causa de morte em indivíduos HbSS (Lanzkron et al. 2008).

Embora o efeito indireto da hidroxiuréia sobre a necrose pulpar assintomática não tenha sido significativo, ele apresentou uma CFP alta de -0.351, com via indireta que passou pela redução da latente comorbidade (Tab. 4). Em conjunto, esses resultados reforçam a importância desse fármaco na melhoria do quadro clínico da anemia falciforme. A hidroxiuréia além de elevar os níveis de HbF, age como doador do vasodilatador óxido nitrico *in vivo* e reduz a contagem de leucócitos, células que participam de forma significativa na oclusão física dos vasos. Desta forma, o uso de hidroxiuréia previne sérias complicações da anemia falciforme, como os eventos de falcização e vasoclusão, precursores das comorbidades associadas a doença (Cançado 2007) e, por conseguinte, da necrose pulpar assintomática.

A prevalência de necrose pulpar assintomática em dentes hígidos de indivíduos HbSS diminuiu com a idade (Tab. 4), o que poderia parecer um contrassenso, considerando que com o envelhecimento o indivíduo estaria mais susceptível as alterações pró-inflamatórias da doença; sendo plausível que houvesse um aumento da prevalência da necrose pulpar assintomática com o avançar da idade. Esse achado poderia ser explicado pela possibilidade dos indivíduos HbSS mais velhos apresentarem menor probabilidade de ter dentes hígidos, o que diminuiu a casuística de necrose pulpar assintomática em dentes hígidos nessa população (Costa et al. 2013). Essa hipótese foi confirmada por Costa et. al (2016), quando utilizaram dados dos prontuários de todos os pacientes HbSS atendidos nesse mesmo centro de referência para avaliar quantitativamente os tipos de procedimentos odontológicos realizados nesses indivíduos, mostrando que o número de dentes restaurados, extraídos, tratados periodontalmente e endodonticamente aumentavam com a idade, o que reduziu a probabilidades de ter dentes hígidos com necrose pulpar assintomática com o aumento da idade. Ademais, acredita-se que a maior taxa de mortalidade de indivíduos HbSS com NPA possa ter contribuído para esse achado (Platts et al. 1994).

Como limitação, é possível ter havido viés de memória, já que o Nº CFs/ano foi auto-relatado; porém, como as crises falcêmicas produzem sinais e sintomas característicos que podem deixar sequelas, acreditamos ser difícil que o indivíduo HbSS não saiba informar o número de crises em que foi acometido no último ano.

Outra limitação do estudo foram as perdas devido à falta de localização dos participantes, o que representou 22.1% do baseline da coorte (n = 25); entretanto essas perdas foram inferiores a 30%, considerado aceitável em estudos longitudinais (Rothman et al. 2016). Dessa forma, para aumentar o poder do estudo e a precisão das estimativas, foram incluídos 59 novos participantes à coorte inicial, a partir de sorteio casual simples das listas de referência da HEMOMAR.

Como atributos positivos desse estudo, destaca-se que todas as variáveis foram homogêneas entre os grupos, exceto Nº CFs/ano, o que reduz o risco de viés de confundimento, tornando os grupos deste estudo comparáveis. Além disso, a amostra proveio da mesma fonte, mesmo os novos participantes foram aleatorizados, o que minimiza o potencial de tendenciosidade que poderia surgir na seleção dos participantes do estudo.

Destaca-se ainda o caráter original do estudo quanto à possível explicação dos mecanismos biológicos envolvidos na associação da NPA em dentes hígidos de indivíduos HbSS por Modelagem de Equações Estruturais, que nos permitiram avaliar simultaneamente diferentes caminhos e o efeito de mediação de algumas co-variáveis a partir de um modelo teórico (Hasman 2015).

Concluiu-se que comorbidades, principalmente as lesões osteoarticulares, aumenta a NPA em dentes hígidos de indivíduos falcêmicos. Além disso, a NPA pode ser considerada um indicador de letalidade da AF.

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3. CAPÍTULO II

HÁ NECESSIDADE DE TRATAR NECROSE PULPAR EM DENTES HÍGIDOS DE INDIVÍDUOS COM ANEMIA FALCIFORME? UM ESTUDO TRANSVERSAL ANINHADO A UMA COORTE

(a ser submetido ao *Journal of Endodontic*)

Resumo

Introdução: A Anemia Falciforme é um potencial fator de risco para Necrose Pulpar Assintomática (NPA) em dentes permanentes hígidos. O objetivo desse estudo foi avaliar a necessidade de tratamento endodôntico da NPA em dentes hígidos de indivíduos falcêmicos por meio dos parâmetros clínicos, imagenológicos e microbiológicos.

Métodos: Este é um estudo transversal aninhado a uma coorte. No primeiro seguimento da coorte foram selecionados dez indivíduos falcêmicos que apresentaram pelo menos um dente com coroa hígida e diagnóstico clínico de NPA obtido por meio da oximetria de pulso adaptada a Odontologia e do teste de sensibilidade pulpar ao frio (n=27 dentes). Alterações na câmara pulpar, raíz e ligamento periodontal (LP) foram identificadas em análises tomográficas. Cultura bacteriana, coloração para bactérias vivas e mortas e qPCR com primers para região 16S rRNA foram utilizados para identificar a presença de bactérias. O microbioma foi determinado pelo sequenciamento MiSeq (Illumina, San Diego, CA). As diferenças de prevalência de NPA entre as co-variáveis do estudo foram identificadas pelo teste exato de Fisher (α=0.05).

Resultados: O diagnóstico de NPA foi confirmado clinicamente em 81.5% dos dentes, com maior prevalência nos maxilares superiores (p=0.016). Nódulo pulpar, hipercementose e espessamento do LP $\leq$ 0.5 mm foram observadas em 14% destes dentes. Quantidades de DNA bacteriano inferior a 100 cópias/µL foram detectadas em 23% dos dentes com NPA. Trinta e uma espécies bacterianas foram identificadas.

Conclusão: As avaliações clínica prospectiva, imagenológica e microbiológica revelam que não há necessidade do tratamento endodôntico da NPA em dentes hígidos de indivíduos falcêmicos

Palavras-chave

Necrose pulpar dental, Anemia Falciforme, Microbiologia

Significância: A significância clínica deste estudo consiste na necessidade de alertar ao cirurgião-dentista de que não há necessidade de tratamento endodôntico da necrose pulpar assintomática em dentes hígidos de indivíduos com anemia falciforme. Para estes dentes recomenda-se a proservação periódica clínica e imagenológica como protocolo de prevenção de infecção de origem dental e suas complicações nesta população.

INTRODUÇÃO

A anemia falciforme (AF) é uma hemoglobinopatia autossômica recessiva não ligada ao sexo, peculiar aos afrodescendentes (1). Sua etiologia ocorre pela mutação de ponto no gene da globina-β da hemoglobina, originando uma molécula alterada (HbS) (2). Em situações persistentes de baixa tensão de oxigênio, as HbS sofrem polimerização, ocasionando a falcização das hemácias (3).

As manifestações clínicas da AF decorrem do aumento da aderência das hemácias falcizadas ao endotélio vascular, por conseguinte, vasoclusão local, episódios de dor e lesão de órgãos vitais (3). Na cavidade bucal pode ocorrer necrose pulpar assintomática (NPA) em dentes hígidos (4), neuropatia do nervo mentoniano e osteomelite mandibular (5). A NPA em dentes hígidos é considerada a principal manifestação bucal desta doença (6-8). Costa, Thomaz e Souza (4) estimaram que o risco relativo de ocorrência dessa manifestação é 8.33 vezes maior em indivíduos com AF (genótipo HbSS) do que naqueles sem a doença. Entretanto, pouco se sabe sobre a necessidade de tratamento desses dentes.

Assim, o objetivo deste estudo foi avaliar a necessidade de tratamento endodôntico em dentes hígidos com NPA em indivíduos HbSS por meio dos parâmetros clínicos, imagenológicos e microbiológicos. Portanto, a hipótese testada foi que há necessidade da realização do tratamento endodôntico.

METODOLOGIA

Desenho do estudo

Trata-se de um estudo transversal aninhado a uma coorte (4). O estudo de coorte teve duas fases: uma retrospectiva e outra prospectiva. A fase retrospectiva foi realizada entre junho de 2010 e junho de 2011 na Supervisão de Hematologia e Hemoterapia do Maranhão (HEMOMAR), São Luís, MA, Brasil, após aprovação do Comitê de Ética em Pesquisa (CEP) local sob protocolo n° 23115004993/2010-71. Nesta fase, os participantes do estudo foram selecionados aleatoriamente pela condição de exposição (anemia falciforme – AF), diagnosticada por meio da eletroforese da hemoglobina ao nascimento. Essa informação foi obtida nos prontuários eletrônicos da HEMOMAR. Os critérios de elegibilidade foram ser maior de 16 anos e ter pelo menos um dente hígido, sem histórico de parestesia do nervo alveolar inferior nos últimos seis meses, sem história prévia de traumatismo dentário e sem doença periodontal. O diagnóstico do desfecho (necrose pulpar assintomática – NPA) foi obtido por meio da oximetria de pulso (OP) adaptada à Odontologia e do teste de sensibilidade pulpar (TSP) ao frio (4).

A fase prospectiva ocorreu no período de setembro de 2015 a setembro de 2016 (protocolo CEP n° 57953814.9.0000.5087). Todos os indivíduos HbSS do estudo (n=113) foram convidados a participar do primeiro seguimento da coorte. Nesta fase, foram submetidos aos mesmos critérios de elegibilidade da fase retrospectiva.

Seleção dos participantes

Dos 113 indivíduos, 81 retornaram para reavaliação. Para completar a amostra foram selecionados por meio de sorteio casual simples do cadastro da Assistência Social da HEMOMAR 64 novos participantes, totalizando 145 participantes. Desse total, foram identificados 20 indivíduos HbSS com NPA que foram convidados a participar deste estudo transversal. Sete indivíduos não aceitaram fazer o exame tomográfico. Dos trezes restantes, três se recusaram a ser submetidos ao tratamento endodôntico. Assim, dez indivíduos participaram deste estudo, com um total de 32 dentes hígidos com NPA. Após avaliação das tomografias cinco dentes foram excluídos devido a presença de calcificação pulpar. Dessa forma, 27 dentes foram submetidos à coleta microbiológica e tratamento endodôntico (Figura 1).

Variáveis do estudo

Neste estudo transversal a exposição foi a NPA e o desfecho principal foi a necessidade de tratamento endodôntico da NPA em dentes hígidos avaliada por meio de parâmetros clínicos (sintomatologia clínica e características macroscópicas da órgão pulpar), imagenológicos (alterações na cavidade pulpar, raiz e ligamento periodontal) e microbiológicos (identificação da presença de bactérias, extração e sequenciamento de DNA bacteriano).

As co-variáveis foram a cor auto-referida (branca, parda ou preta), sexo (masculino ou feminino), idade (em anos) e maxilares (superior ou inferior).

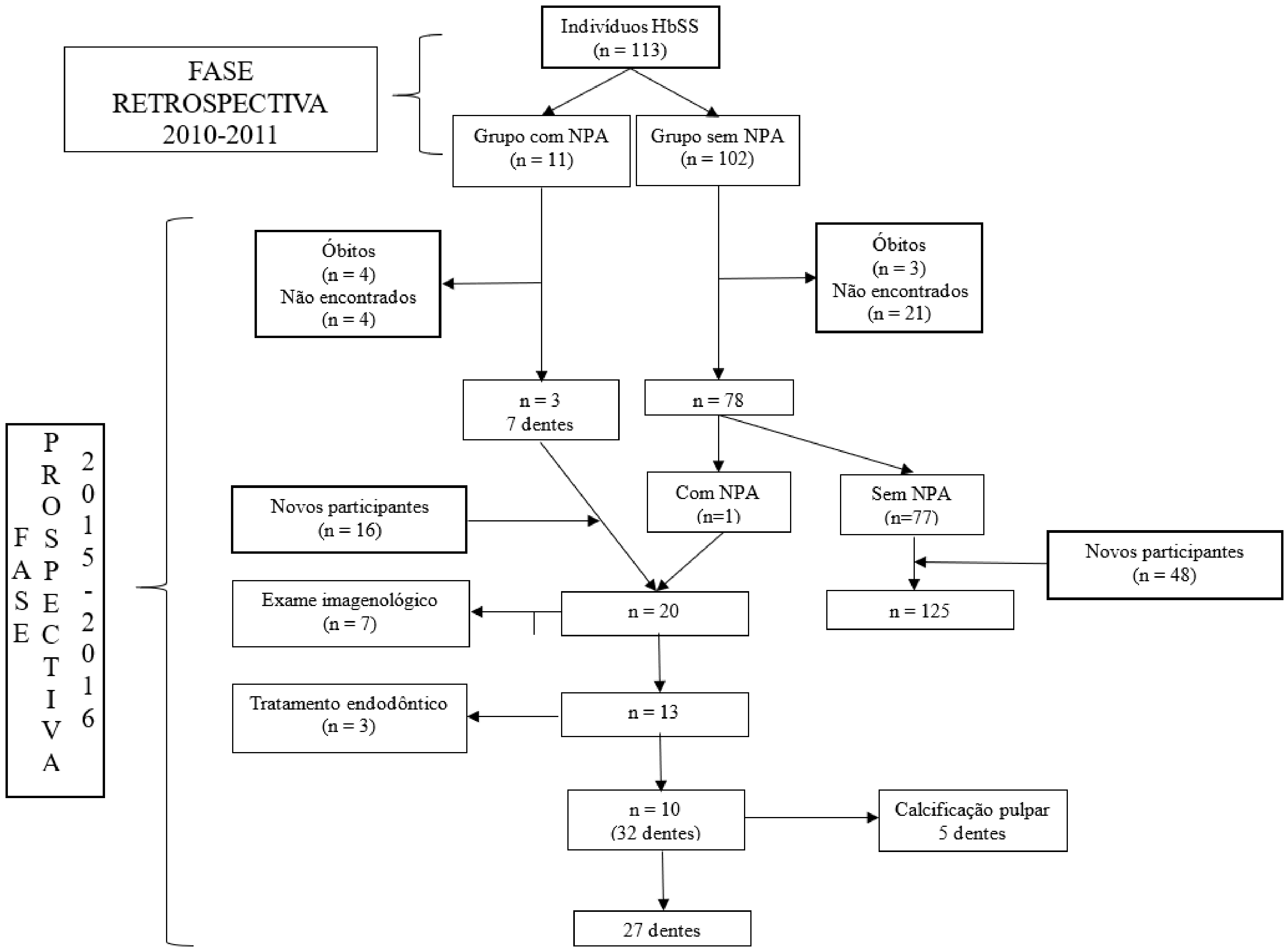

Figura 1. Fluxograma do seguimento e seleção dos novos participantes do estudo.

Tamanho amostral

Estimou-se que uma amostra de 27 dentes hígidos teria o poder de 99.9% para identificar diferenças entre as proporções de presença de DNA bacteriano entre os grupos com e sem NPA, considerando alpha de 5%, teste bicaudal, proporção de 1:4 entre dentes com e sem NPA. Admitiu-se uma proporção de 1% de dentes hígidos com DNA bacteriano e 95,45% de DNA bacteriano em dentes com NPA.

Diagnóstico da condição pulpar e periapical

Parâmetros clínicos

Os dentes hígidos de indivíduos HbSS foram reavaliados simultaneamente pela OP adaptada a Odontologia e pelo TSP ao frio. Ambas as mensurações foram realizadas com isolamento relativo. Primeiro foi realizada a OP e cinco minutos depois, o TSP ao frio.

A %SaO_2 corpórea e pulpar foram mensuradas com ajuda do oxímetro de pulso MD300A (IMFtec Tecnologia para Saúde LTDA, São Paulo, SP, Brasil). O sensor desse aparelho foi adaptado para uso odontológico e já empregado na fase retrospectiva da coorte (4). Cada sensor possui dois diodos. Durante a mensuração da %SaO_2 pulpar, os diodos foram mantidos paralelos entre si, para que o diodo receptor recebesse a luz transmitida através do dente. Para não influenciar a leitura do oximetro, o paciente foi orientado a não movimentar a cabeça e o refletor da cadeira odontológica foi desligado.

As mensurações foram realizadas inicialmente no dedo indicador direito do paciente (%SaO_2 corpórea) e, em seguida, no centro das faces vestibulares e palatal/lingual dos dentes (%SaO_2 pulpar). O teste foi repetido três vezes e a mediana dos valores foi utilizada. Os dentes foram considerados vitais quando apresentaram mediana da %SaO_2 pulpar ≥ 79% (10), ou com NPA quando o OP não registrava a leitura da %SaO_2 pulpar (4).

A resposta ao TSP ao frio foi registrada como positiva ou negativa. O gás diclorodifluormetano (-50°C; Endofrost, Roeko, Langenau, Alemanha) foi aplicado no centro do terço cervical da face vestibular do dente com ajuda de um microbrush. Cada paciente foi orientado a levantar o antebraço e a mão esquerda imediatamente após apresentar sensibilidade (resposta positiva). A NPA foi constatada quando o paciente não apresentava sensibilidade ao teste (resposta negativa).

Além disso, os seguintes dados clínicos foram registrados: a) história pregressa de dor (ausente ou presente); b) resposta a percussão vertical (positiva ou negativa); c) dor a palpação dígito-apical (ausente ou presente); d) mobilidade dentária (normal ou aumentada).

Parâmetros imagenológicos

Foram realizadas tomografias computadorizadas cone beam utilizando-se tomógrafo de feixe cônico Prexion 3D XP68 (PreXion, Inc., San Mateo, CA, USA) 87.24 KpV e 4 mA. As imagens foram visualizadas pelo programa Prex Viewer (PreXion, Inc., San Mateo, CA, USA). As análises foram feitas por duas examinadoras treinadas e calibradas (Kappa=0.91) na tela das reformatações multiplanares de acordo com os critérios de Lopes et al. (9). Foram investigadas alterações : na cavidade pulpar (sem alteração, atresia, calcificação da câmara pulpar e/ou do canal radicular, nódulos pulpares); na raiz (sem alteração, reabsorção externa lateral e/ou apical, reabsorção interna, hipercementose e outros); e no ligamento periodontal (LP), de acordo o Índice Periapical de Tomografia Computadorizada Cone Beam (IPTCCB) de Estrela et al. (10) (sem alteração, espessamento parcial ou total, perda parcial ou total).

Características macroscópicas do órgão pulpar

Após realização do isolamento absoluto e rigorosa antissepsia do conjunto dente/grampo/lençol de borracha com solução de hipoclorito de sódio 2,5% foi feito o acesso à cavidade pulpar com brocas estéreis.

Na sequência foi confirmado o diagnóstico da condição pulpar pela inspeção visual das características macroscópicas do tecido pulpar quanto ao sangramento, coloração, consistência e resistência ao corte, assim classificada: polpa viva – PV (sangramento normal, tecido pulpar com coloração róseo-avermelhada, consistente e resistente ao corte), polpa em decomposição – PD (sangramento discreto, pequena quantidade de exsudato róseo, tecido pulpar com coloração esbranquiçada, consistência pastosa e sem resistência ao corte) e NPA (câmara pulpar vazia ou tecido pulpar totalmente desidratado semelhante à "palha") (11). PD e NPA foram considerados "com NPA" e PV "sem NPA".

Coleta das amostras

O canal radicular foi preenchido com soro fisiológico e foi coletado 0.5 µL desse lavado. Tanto a irrigação quanto a aspiração foram feitas com seringas hipodérmicas. Em seguida, uma lima endodôntica tipo K#15 ou #20 (Maillefer, Ballaigues, Suiça) foi introduzida até 1 mm aquém do vértice radicular (medida obtida da tomografia), e aplicado um discreto movimento no sentido horário até a lima ficar justa no canal, e em seguida foi removida para coletar raspas de dentina.

Na sequência, duas pontas de papel absorventes foram colocadas na mesma medida para absorver o fluído do canal. Cada ponta de papel foi mantida na posição por um minuto. A lima com o cabo removido, e as pontas de papel foram transferidas para tubos de Eppendorf (1.5 mL) estéreis individuais contendo reagente RNA bacteria protect (Qiagen, Hilden, Alemanha), conforme instruções do fabricante. As amostras foram armazenados a -20°C para serem utilizadas na cultura bacteriana e fluorescência. As amostras de dentina coletadas com auxilio de limas e o fluido absorvido pelos cones de papel foram empregados na extração de DNA bacteriano para ser utilizado na Reação em Cadeia de Polimerase em tempo real (qPCR).

Para o controle positivo da qPCR foram coletadas amostras bacterianas de dois dentes com diagnóstico de necrose pulpar por cárie de dois indivíduos HbSS participantes do estudo, com a câmara pulpar exposta ao meio bucal e lesão periapical >8mm de acordo com Estrela et al. (10), IPTCCB igual a 5, onde sabidamente seriam encontradas bactérias nos canais radiculares,

O preparo do canal foi realizado empregando-se instrumentação manual ou reciprocante (Motor VDW Silver – VDW Gmbh, Bayerwaldstr, Monique, Alemanha). A obturação dos canais radiculares foi realizada com as técnicas de condensação lateral ou do cone único e cimento à base de resina epóxica/hidróxido de cálcio (AH Plus™, Dentisplay, Petropólis, RJ, Brasil). Todos os canais radiculares foram submetidos ao protocolo de tratamento em duas sessões, mantendo-se a medicação intracanal à base de hidróxido de cálcio (Callen™, SS White, Rio de Janeiro, RJ, Brasil) por 14 dias.

Análise laboratorial

A detecção das bactérias foi realizada por meio de cultura bacteriana, coloração para bactérias vivas e mortas, técnica de biologia molecular de DNA por qPCR e determinação do microbioma.

Cultura bacteriana

Diluições decimais (até 10^{-3}) em PBS (*Phosphate Buffered Saline* – Tampão Fosfato-Salino) do lavado foram espalhadas sobre a superfície de placas contendo Ágar de Sangue Aneoróbico (CDC) para microrganismos anaeróbios com 5% de sangue de carneiro (Becton, Dickinson and Company, Franklin Lakes, NJ, EUA) de acordo com Sato et al. (12). As placas foram incubadas em anaerobiose a 37°C por sete dias.

Coloração para bactérias vivas e mortas

As amostras foram submetidas a diluições seriadas decimais (até 10^{-3}). Para verificar a presença de bacterias vivas e mortas foi utilizado o kit LIVE/DEAD® *Bac*Light™ (Life Technologies, Carlsbad, CA, EUA). A análise foi realizada em microscópio de epifluorescência (Axio Imager, Zeiss, Oberkochen, Alemanha).

qPCR

O DNA de cada amostra foi extraido usando o *Qiamp DNA Mini Kit* (Qiacube, Qiagen, Hilden, Alemanha), amplificado por meio de primers específicos da região 16S rRNA (*Forward*: 5'-ACTCCTACGGGAGGCAGCAG-3' e *reverse*: GTATTACCGCGGCTGCTGGCAC) (13) e purificado utilizando o kit wizard SV 96 PCR clean-Up System (Promega, Madison, Wisconsin, EUA).

Amostras de DNA extraído das amostras foram amplificadas pela qPCR juntamente com o plasmídeo recombinante (controle interno). Os ensaios foram realizados em aparelho QuantStudio™ 6 Flex (Life Technologies, Carlsbad, CA, EUA).

Determinação do microbioma

As amostras foram sequenciadas pela empresa Neoprospecta (Florianópolis, Santa Catarina, Brasil) para determinação do microbioma endodôntico de dentes com NPA em indivíduos HbSS pelo método de síntese com os primers V3-V4 341F-806R para o gene 16S rRNA no sequenciador de última geração MiSeq (Illumina, San Diego, California, EUA). Para as análises computacionais das sequências os amplicons obtidos do sequenciamento foram analisados na Plataforma MiSeq (Illumina, San Diego, California, EUA). As seqüências agrupadas foram submetidas à classificação de taxonomia comparando-as com o banco de dados 16S rRNA da empresa (NeoRefdb, Neoprospecta Microbiome Technologies, Brasil). As seqüências com pelo menos 99% de identidade no banco de dados de referência foram atribuídas por taxonomia (14).

Análise Estatística

Foram estimadas frequências absolutas e relativas para as variáveis categóricas e média (± desvio-padrão) ou mediana (± desvios interquartílico) para as variáveis numéricas. Diferenças de prevalência de NPA entre as co-variáveis cor, sexo, idade e maxilares foram identificadas pelo teste exato de Fisher. O nível de significância estabelecido foi de 5%. As análises foram realizadas por meio do programa Stata versão 9.0 (Stata Corp., College Station, Texas, EUA).

RESULTADOS

A amostra do estudo foi composta por 63.2% indivíduos da cor parda, 18.2% branca e 18.2% preta, 72.7% do sexo feminino e 27.3% masculino, com idade média de 39.7(±19.6) anos. A NPA apresentou associação significante com os maxilares superiores (p=0.016).

Todos os dentes hígidos diagnosticados com NPA pela OP e TSP ao frio apresentaram história pregressa (baseline) e atual (seguimento) de ausência de sintomatologia. Os testes de palpação dígito-apical, percussão vertical e mobilidade dental apresentaram resposta negativa.

A Tabela 1 apresenta as características macroscópicas da polpa, alterações imagenológicas da câmara pulpar, raiz e ligamento periodontal, análises microbiológicas e a quantificação de DNA bacteriano por meio de qPCR. A hipótese diagnóstica de NPA foi confirmada clinicamente em 81.5% dos dentes (22/27; Tabela 1). Foram identificadas alterações imagenológicas em 14% dos dentes com NPA (3/22; Tabela 1). Não houve crescimento da cultura bacteriana nem identificação de bacterias pelo microscópio de epifluorescência . Em 23% dos dentes com NPA (5/22; Tabela 1) foi detectada uma quantidade de DNA bacteriano inferior a 100 cópias/µL. A Figura 3 mostra a diversidade do microbioma por amostra. Foram identificadas as espécies *Acinetobacter calcoaceticus, Acinetobacter nosocomialis, Agrobacterium tumefaciens, Bifidobacterium breve, Brevundimonas vesicularis, Streptococcus parasanguinis, Escherichia coli, Granulicatella adiacens, Lactobacillus ssp., Macrococcus caseolyticus, Moraxella* *osloensis, Proteus mirabilis e Pseudomonas putida* nos dentes da amostra (Figura 3).

Tabela 1 - Parâmetros clínicos, imagenológicos e microbiológicos da condição pulpar e periapical, segundo o paciente, idade e dentes.

Pacientes	Idade	Dentes (n=27)	Características clínicas†	Aspectos imagenológicos♦	Cultura bacteriana	Fluorescência	qPCR cópias/µL
01	37	21	NPA	-	-	-	-
		12	NPA	-	-	-	-
02	25	25	NPA	-	-	-	+*
		23	NPA	-	-	-	+*
		13	PD	espessamento do LP ≤ 0.5mm hipercementose	-	-	-
		45	PV	-	-	-	-
		35	PV	-	-	-	-
		15	PD	-	-	-	-
		33	PD	-	-	-	-
		46	**necrose pulpar**	**lesão periapical > 8.0mm**	+	+	+
03	51	33	PV	-	-	-	-
04	47	33	PV	-	-	-	-
		23	PD	-	-	-	-
		16	NPA	-	-	-	-
		24	PD	-	-	-	-
		17	**necrose pulpar**	**lesão periapical > 8.0mm**	+	+	+
05	17	45	NPA	espessamento do LP ≤ 0.5mm	-	-	+*
		35	NPA	espessamento do LP ≤ 0.5mm nódulo pulpar	-	-	-
06	36	27	NPA	-	-	-	+*
		21	NPA	-	-	-	+*
		45	PD	-	-	-	-
		33	PD	-	-	-	-
07	17	13	NPA	-	-	-	-
		34	PD	-	-	-	-
08	17	42	PV	-	-	-	-
		32	PD	-	-	-	-
09	68	24	NPA	-	-	-	-
		45	NPA	-	-	-	-
10	70	43	NPA	-	-	-	-

qPCR=PCR em tempo real. NPA=Necrose Pulpar Assintomática. PD=Polpa em decomposição. PV=Polpa Viva. †NPA = foram agrupados os dentes com polpa em decomposição e necrose pulpar assintomática. ♦Alterações imagenológicas = foram consideradas as alterações da câmara pulpar, da raiz e ligamento periodontal. LP=Ligamento Periodontal. (-) negativo. (+) positivo. Dados dos dentes controles positivos em negritos. *<100 cópias/µL.

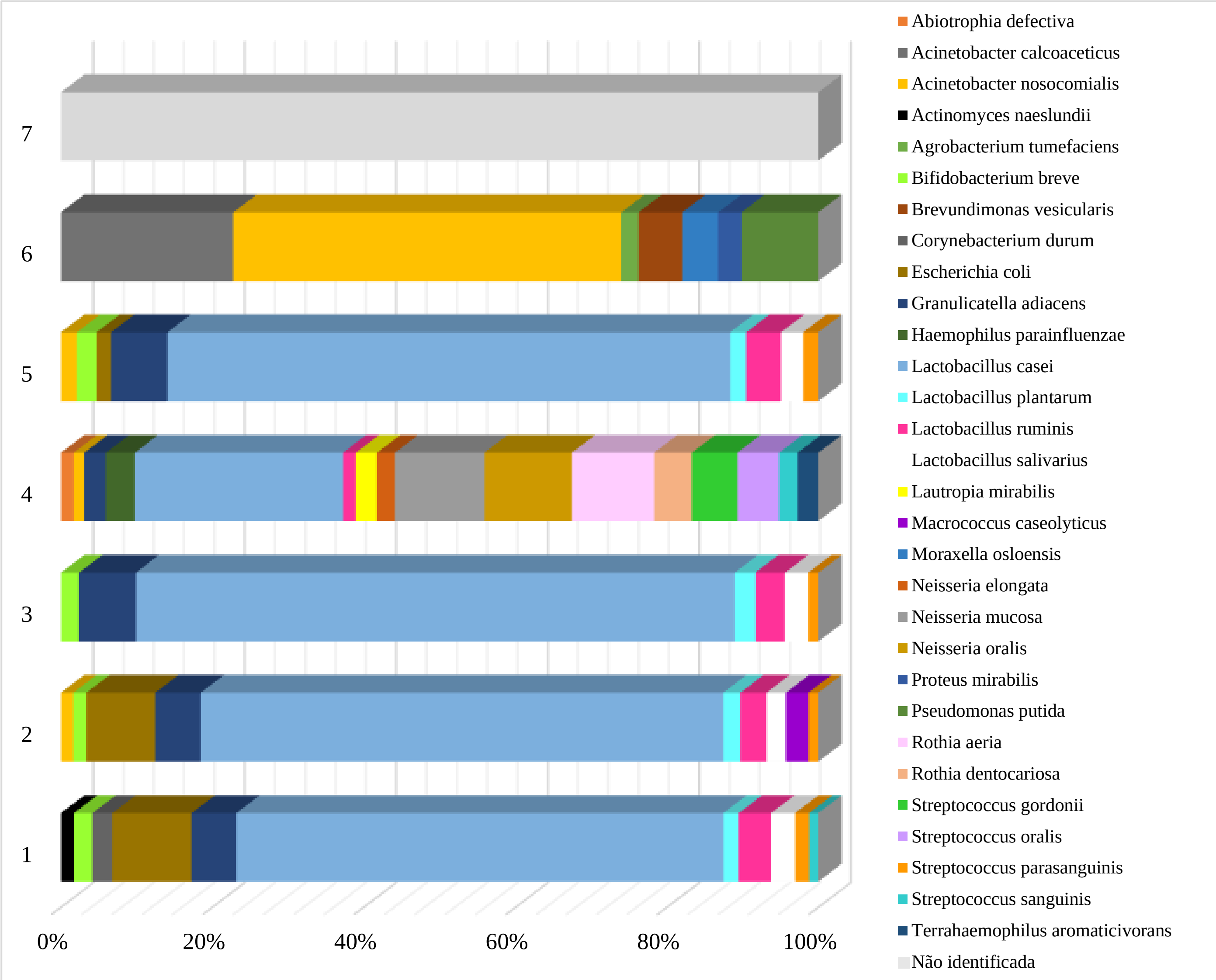

Figura 2 – Percentual da abundância relativa de espécies por amostras. Amostras 1 e 4 = necrose pulpar por cárie (controles positivo). Amostras 2, 3, 5, 6 e 7 = NPA em dentes hígidos.

DISCUSSÃO

A hipótese testada neste estudo foi rejeitada, isto é, dentes hígidos com NPA sem lesão periapical em pacientes falcêmicos não necessitam de tratamento endodôntico. A pequena quantidade de DNA bacteriano detectado em cada amostra, a constatação de que as bactérias identificadas não são comuns nas infecções endodônticas primárias, e a observação de que sete dentes com diagnóstico de NPA de três indivíduos da coorte de base (26% da amostra) apresentaram silêncio clínico durante todo o período de prospecção deste estudo (6 anos) sustentam tal afirmativa. Apenas três destes dentes (pacientes 02 e 05) apresentou espessamento do ligamento periodontal ≤ 0.5mm, o que não é considerado lesão periapical (Tabela 1) (10).

Kakehashi, Stanley e Fitzgerald em modelo animal experimental “germ free” em ratos (15) e Möller et al. em macacos (16) evidenciaram a importância do papel dos microrganismos e seus subprodutos para induzir infecções pulpares e periapicais. Tal evidência foi confirmada por Sundqvist (17) quando avaliou 32 canais de dentes humanos uniradiculares com coroas hígidas e necrose pulpar em decorrência de trauma dentário e constatou que apenas 18 dentes apresentaram lesões periapicais, confirmando a importância da presença de microrganismos na origem e manutenção das infecções endodônticas. No presente estudo a quantidade de DNA bacteriano identificada por qPCR em 23% dos dentes foi inferior a 100 cópias/µL por amostra (Tabela 1). Estudos prévios mostram que dentes com essa carga bacteriana não desenvolveram infecção endodôntica (19-22).

Foi observado semelhança da diversidade da composição bacteriana dos dentes necrosados hígidos (#23 e #25) com as do dente controle (#46) no paciente 02 (Tabela 1). Dentre as espécies bacterianas identificadas, apenas a *Granulicatella adiacens* pode ser encontrada, com baixa frequência, nas infecções endodônticas primárias. Devido a alta sensibilidade da análise por qPCR, acreditamos que pode ter ocorrido contaminação de saliva no momento do acesso a câmara pulpar. Isso reforça a suposição de que a NPA nesses indivíduos ocorra por infarto anêmico devido a isquemia tecidual durante as crises vasoclusivas, levando o órgão pulpar a necrose por coagulação das proteínas teciduais, semelhante ao que ocorre nos casos de traumatismos dentários (luxação, avulsão e intrusão), quando o aporte sanguíneo pulpar via forame apical é bloqueado. A polpa, assim necrosada, se apresenta seca, semelhante a "palha" e sem contaminação bacteriana (23). Entretanto, não descartamos a possibilidade de que a polpa durante o processo de coagulação possa atrair por via hematogênica bactérias oriundas ou não de infecções em outros órgãos vitais, mas em quantidade insuficiente para estabelecer infecção pulpar e lesões periapicais. Esse fenômeno pode ser confirmado em um recente estudo no qual foi observado DNA bacteriano detectável em polpas de dentes hígidos de pacientes saudáveis (24). A infecção endodôntica por via anacorética (25-27) poderia explicar a presença de bactérias em dentes hígidos com NPA (25). No presente estudo foram detectadas alguns microorganismos responsáveis por infecções sistêmicas comuns em indivíduos HbSS (28-29), como o *Acinetobacter ssp.* (septicemia), *Escherichia coli* (infecção do trato urinário e septicemia), *Proteus mirabilis* (infecção do trato urinário) e *Streptococcus ssp.* (septicemia). Contudo, esses microorganismos podem estar presente transitoriamente como parte da microbiota oral (24).

Cinco dentes foram excluídos da amostra por apresentarem calcificações pulpares identificadas nos exames tomográficos, o que ressalta a importância do exame de imagem para aumentar a especificidade do diagnóstico da condição pulpar. As calcificações podem ocorrer como consequência da resposta pulpar à hipóxia passageira e redução do seu metabolismo, quando ocorre lesões vasculares no terço apical, semelhante aos casos de traumatismo dentário (concussão e subluxação). Nestes casos, quando ocorre lesão parcial do feixe vascular e nervoso, sem que tenha havido interrupção total do fluxo sanguíneo, as células pulpares podem sofrer metaplasia, se transformam em odontoblastos que iniciam uma produção aleatória e desorganizada de dentina reacional depositada em todo o espaço pulpar, obliterando-o totalmente, caracterizando a metaformose cálcica da polpa (23).

Cinco dentes inferiores (dois pré-molares, dois caninos e um incisivo lateral) apresentaram polpa viva constatada após o acesso a cavidade pulpar, apesar da resposta negativa aos testes de OP e TSP ao frio. Entretanto, ressaltamos que os dados semiotécnicos são apenas parâmetros para se estabelecer as hipóteses diagnósticas da condição pulpar. O teste padrão-ouro para a confirmação dessas hipóteses é a inspeção visual por meio da avaliação macroscópica da polpa. Isso poderia justificar os diagnósticos de NPA falso-positivos, considerados uma limitação deste estudo. A ausência da sensibilidade de pré-molares, caninos e incisivos inferiores aos estímulos térmicos nestes indivíduos pode ocorrer pela compressão do nervo mentoniano por trombos na artéria alveolar inferior ocasionado pelas crises vasoclusivas, comuns na anemia falciforme (29). A resposta negativa para a %SaO_2 pulpar pode ser atribuída a hipóxia tecidual após vasoclusão da artéria mentoniana (30).

Neste estudo a NPA foi mais prevalente nos dentes superiores. Segundo Souza, Thomaz e Costa (31) os níveis de %SaO_2 pulpar nos dentes vitais de indivíduos HbSS são mais baixos na maxila, provavelmente devido ao maior acúmulo de hemácias falcizadas nos amplos espaços maxilares medulares, o que poderia ser considerado um fator predisponente para a NPA.

Até o presente momento esse é o primeiro estudo a avaliar os parâmetros clínicos, imagenológicos e a presença de bactérias em dentes hígidos com NPA em indivíduos HbSS e a apontar evidências que suportam a conclusão de que não há necessidade de tratamento endodôntico destes dentes, já que não há evidências de que o tratamento endodôntico traria benefícios para melhoria de qualidade de vida destes indivíduos. Entretanto, recomenda-se a proservação periódica clínica e imagenológica destes dentes, no minímo a cada seis meses, como protocolo de prevenção de infecção de origem dental nesta população.

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4. CONSIDERAÇÕES FINAIS

Esta TESE constitui-se numa contribuição original para o estado da arte do conhecimento sobre as manifestações bucais da AF de interesse odontológico.

Os resultados do primeiro estudo – MECANISMOS BIOLÓGICOS ASSOCIADOS A NECROSE PULPAR ASSINTOMÁTICA DE INDIVIDUOS COM ANEMIA FALCIFORME: UM ESTUDO DE COORTE PROSPECTIVO– apontam para a necessidade de alertar aos cirurgiões-dentistas que pacientes falcêmicos com lesões osteoarticulares podem apresentar NPA em dentes hígidos. Essas duas condições associadas sinalizam a severidade da doença e podem ser consideradas um indicador de letalidade da AF.

Entretanto, os resultados do segundo estudo – HÁ NECESSIDADE DE TRATAR DENTES HÍGIDOS COM NECROSE PULPAR ASSINTOMÁTICA DE INDIVIDUOS COM ANEMIA FALCIFORME? – mostram que a NPA em dentes hígidos sem evidências clínicas e imagenológicas de infecção endodôntica e lesão periapical não apresentaram carga bacteriana suficiente para estabelecer uma infecção endodôntica , e por isso não necessitam de tratamento.

Essa recomendação também está respaldada no seguimento do estudo de coorte em que, após seis anos, confirmamos que não ocorreu infecção endodôntica nem a presença de lesão periapical em dentes com diagnóstico clínico de NPA.

Portanto, acreditamos que a partir desses novos conhecimentos podemos ousar propor às diretrizes para atendimento odontológico aos pacientes com AF que:

1) Pacientes com AF e lesões osteoarticulares necessitam que seja investigada a presença de NPA;

2) Dentes hígidos com diagnóstico de NPA sem evidências clínicas e imagenológicas de infecção endodôntica e lesão periapical não necessitam de tratamento endodôntico;
3) Esses dentes devem ser proservados periodicamente a cada 6 meses.

A adoção desses critérios possibilitará aos cirurgiões-dentistas estabelecer o diagnóstico preciso da condição pulpar e à tomada de decisão terapêutica segura para prevenir infecção de origem dental nessa população.

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Association between Sickle Cell Anemia and Pulp Necrosis

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Abstract

Introduction: The purpose of this study was to evaluate the association between sickle cell anemia (SCA) and pulp necrosis (PN). **Methods:** One hundred thirteen individuals with SCA (ie, the exposed group) from the Supervision of Hematology and Hemotherapy of Maranhão (HEMOMAR), Maranhão, Brazil, and 226 individuals without SCA, the sickle cell trait, or other diseases (ie, the nonexposed group) were enrolled in this study. All participants were over 16 years old and had at least 1 clinically intact permanent tooth. Patients with SCA and a history of lower alveolar nerve paresthesia and those who had suffered from vasoocclusive crises within the previous 6 months were excluded. PN of clinically intact permanent teeth without a history of orofacial trauma was diagnosed using the cold thermal test (CTT) and pulse oximetry adapted for dentistry (POD). Poisson regression was used to estimate the associations ($P < .05$). **Results:** In an unadjusted analysis, the occurrence of PN in clinically intact permanent teeth was 8.33 times higher in the exposed group than in the nonexposed group ($P < .001$). This association remained significant after adjusting for a history of orofacial trauma and folic acid use for the CTT and POD evaluations. **Conclusions:** In conclusion, SCA is a potential risk factor for PN in clinically intact permanent tooth. *(J Endod 2013;39:177–181)*

Key Words

Endodontics, hematology, oral diagnosis, pulse oximetry

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Sickle cell anemia (SCA), the most common genetic disorder worldwide, is a hemoglobinopathy transmitted as an autosomal recessive non–sex-linked disease, which is common among individuals of African descent (1). This disease is caused by a point mutation in the β-globin gene of normal hemoglobin (HbA) resulting in a modified hemoglobin (HbS). The diagnosis of SCA is used for the homozygous form of the disease when a person receives a gene for HbS from each parent. When the subject receives a single gene for HbS from just 1 parent and a gene for HbA from the other, he/she does not have the disease but is a carrier of the sickle cell trait (2).

In situations of persistently low oxygen tension, these cells are recognized by the reticuloendothelial system and are destroyed, thus reducing their average life span. Red blood cells of HbA individuals survive about 120 days, and red blood cells of HbS individuals survive only between 15 and 25 days. The accumulation of distorted cells can cause vasoocclusion, which can lead to anoxia, infarcts, necrosis, and pain. An additional mechanism causing vascular obstruction, the sickle erythrocyte is more adherent than normal red blood cells to the vascular endothelium (3).

The pathological manifestations of this disease can be observed in mineralized and connective tissues of the body such as in the kidneys, liver, heart, and lungs also occur in the oral tissues, but these signs are not pathognomonic of SCA (4). In the oral cavity, the most common clinical manifestations are pallor of the oral mucosa, delayed tooth eruption, cranial deformities, hypomaturation and hypomineralization of enamel and dentin, and calcifications such as denticles in the pulp chamber (5).

The few published epidemiologic studies that exist show a higher prevalence of decay (6) and periodontal disease (7, 8) in patients with SCA. The occurrence of asymptomatic pulp necrosis (PN) in clinically intact permanent teeth has also been suggested in patients with SCA (9–12). The rationale for this oral complication is based on vasoocclusion of the pulp microcirculation because of the accumulation of sickled cells, but only 1 study concluded that SCA is the potential cause of the PN independent of other associated factors. However, this investigation did not estimate this association (12). The aim of this study was to estimate the association between SCA and PN. The hypothesis tested was that there is no association between SCA and PN.

Materials and Methods

This was a cohort study. This study had both retrospective and prospective phases. The exposure (SCA) and the outcome of interest (PN) were already existent at the time of the study, and the allocation of subjects in the study took place from retrospectively investigating for the exposure condition in hospital records. Therefore, the exposure assessment was made based on a past moment; however, the diagnosis of the outcome was made in the present moment. The study was approved by the local research ethics committee (protocol #23115-04993/2010-71).

Only patients with SCA registered in the Social Services Sector of the Supervision of Hematology and Hemotherapy of Maranhão (HEMOMAR), Maranhão, Brazil, in 2000 to 2011 (ie, the exposed group) were enrolled in this study. The nonexposed group was composed of patients' relatives who did not have SCA, the sickle cell trait, or any other systemic condition. The inclusion criteria were age greater than 16 years and the presence of at least 1 clinically intact permanent tooth. Patients with SCA and a history of lower alveolar nerve paresthesia and those who had suffered from vasoocclusive crises within the previous 6 months were excluded. Teeth affected by trauma, caries, or restoration were also excluded from both groups.

The calculation of the sample size was based on the difference in the size of the effect between the exposed and nonexposed groups. The exposed group was made up of 113 individuals with SCA, and the nonexposed group consisted of 226 people without the condition ($N = 339$). This sample size had a power of 80% to identify differences of 5.81% in the frequency of PN between groups. An estimate of 6% of PN was expected among individuals with SCA (12). The level of significance was established at 5%.

Initially, we constructed a list of all SCA patients eligible for the study based on the records of HEMOMAR. Then, a selection of 113 subjects was performed using simple random sampling without replacement. Extra subjects (10%) were randomly selected to replace any who refused, died, or were unable to be contacted ($n = 11$). These people were contacted and invited to visit HEMOMAR to perform a dental examination and answer a questionnaire.

SCA was diagnosed using the electrophoresis technique. HEMOMAR performs extended family screening for this condition as part of a national screening program for congenital diseases. After an examination of each patient with SCA, 2 controls were randomly selected from the records of the family screening.

The main outcome (ie, PN of clinically intact permanent teeth) was determined by pulse oximetry adapted for dentistry (POD) and the cold thermal test (CTT) by 3 calibrated examiners (kappa = 0.9). The vitality of the pulp depends not only on pulpal nerve fibers but also on an extensive network of peripheral blood vessels. Therefore, the conclusive diagnosis of PN in patients with SCA should not be based solely on the findings of testing thermal (hot/cold) and mechanical pulp sensitivity (13). Hence, we also measured the measurement of pulpal blood flow by POD.

The physiometric test conducted by pulse oximetry is a noninvasive method that determines the level of oxygen saturation (SaO_2) and the pulse rate in pulp's tissue. This test has already been validated in human teeth affected by dental trauma (13). Pulse oximetry is based on 2 basic principles: spectrophotometry and plethysmography. A spectrophotometer measures the amount of light transmitted (or reflected) by the capillaries of the patient synchronized with the cardiac pulse, and plethysmography records the volume of arterial blood in the tissue and the light absorption by the blood, which changes with the heartbeat (14).

A pulse oximeter (MD 300A; IMFtech Technology for Health Ltda, São Paulo, SP, Brazil) was used to evaluate POD by measuring the percentage of SaO_2 of the dental pulp. This apparatus consists of a sensor with 2 diodes, a transmitter, and a receiver adapted for human dental anatomy. The percentage of SaO_2 serves as a parameter to assess the condition of the pulp (13).

POD was evaluated in 2 steps. In the first step, the percentage of SaO_2 was measured in the right index finger of each participant. In the second step, the percentage of SaO_2 of the clinically intact permanent tooth was measured. The light reflector of the dentist was turned off so as not to interfere in the signal of the oximeter. The patients were told not to move their heads. Subsequently, a light-emitting diode was placed on the labial side of the tooth and a photodiode on the palatal or lingual side, keeping the 2 diodes parallel. The percentage SaO_2 of the pulp was obtained when the signal stabilized. Teeth for which oximeter readings were not recorded were considered without pulp vitality. The median of the 3 readings was considered.

The CTT was performed 5 minutes after POD. Using a microbrush, dichlorodifluoromethane gas (Endo Frost; Vigodent SA Industria e Comercio, Bonsucesso, Rio de Janeiro, RJ, Brazil) was applied to the cervical portion of the buccal surface of the tooth for 4 seconds. Each participant had been asked to raise his/her left hand immediately upon feeling pain and to lower it as the degree of pain reduced but to keep it raised until the symptoms disappeared completely. PN was considered when the patient was insensitive to the CTT.

The covariables were as follows: self-reported ethnicity classified as white, mulatto, or black according to the Brazilian Institute of Geography and Statistics; sex; age (in years); socioeconomic class categorized as A/B, C, or D/E (15); a history of orofacial trauma (yes or no); and folic acid use (yes or no).

Statistical Analysis

The data were recorded as numbers and were input in 2 Excel (Microsoft Corp, Redmond, WA) spreadsheets with inputting errors being corrected by comparing the spreadsheets. Statistical analyses were performed using the Stata statistics program version 9.0 (Stata Corp, College Station, TX). The Stata transfer software (Stata Corp) was used to export data from Excel to Stata. The prevalence of PN and the 95% confidence interval (CI) were estimated for both groups and compared using the chi-square and Kruskal-Wallis tests ($P < .05$). The Spearman correlation test was used to compare the CTT and POD methods.

The unadjusted and adjusted relative risk (RR) and their respective 95% CIs were estimated in Poisson regression models to evaluate associations between SCA and PN. Two adjusted models were built: one considering PN diagnosed by the CTT (model 1) and the other by POD (model 2). The number of teeth with a diagnosis of PN was summed for each patient in the analysis. A level of significance of 20% ($P < .20$) in the unadjusted analysis was adopted as the selection criterion for covariables to be inserted into the adjusted models as potential confounding factors. The removal of covariables was performed by the step-by-step method, with only variables with $P < .10$ remaining in the final model.

Results

One hundred twenty-four SCA patients were selected for the study; 9 refused to participate and 2 were toothless. All 226 people selected for the nonexposed group agreed to participate. Thus, 10,848 teeth were evaluated. Of these teeth, 8,220 were not considered in the examination of the pulp vitality because they were not clinically intact permanent teeth or were not present in the mouth. Therefore, 2,628 teeth were examined according to both the CTT and POD methods.

Figure 1 summarizes the demographic, socioeconomic, and clinical characteristics of the population. The sample was composed primarily of people from socioeconomic class C (59.9%), blacks (61.6%), women (54.6%) who were not taking folic acid (70.8%), and subjects with no history of orofacial trauma (97.3%). The exposed and nonexposed groups were statistically similar in respect to orofacial trauma ($P = 1.000$), sex ($P = .190$), age ($P = .224$), and socioeconomic class ($P = .257$). However, the use of acid folic was significantly higher in SCA than in the nonexposed group ($P < .001$). There was also a higher frequency of PN among clinically intact permanent teeth in the exposed group compared with the nonexposed group ($P = .005$). This association was the same for the CTT and POD methods.

Only 17 individuals (5%; 95% CI, 2.95–7.91) had at least 1 clinically intact permanent tooth with PN by both diagnostic methods, 11 (64.7%) of whom were in the exposed group. The prevalence of teeth with PN was 18.3% ($n = 62$) according to both methods. Fifty (80.64%) of these teeth occurred in the exposed group.

The variables age, percentage of SaO_2 of the finger, and the number of clinically intact permanent teeth with PN exhibited asymmetric distributions between groups. The median age (ie, 26 years) was similar in both groups. The median percentage SaO_2 of the finger

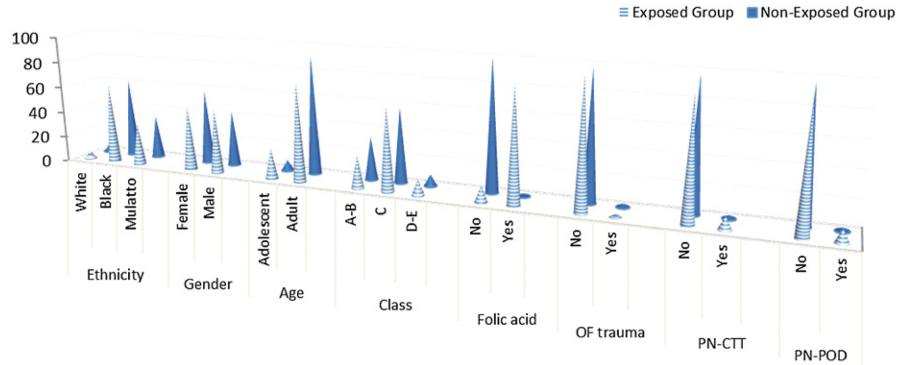

Figure 1. The demographic, socioeconomic, and clinical characteristics of the participants. OF, orofacial.

was higher in the nonexposed group (87%) than in the exposed group (85%, P = .021).

There was a high correlation between the CTT and POD methods in the diagnosis of PN (Spearman test, R = .94; P < .001). The dental groups with the highest means of teeth with PN were the premolars and incisors in both the exposed and nonexposed groups according to both methods. The mean number of teeth with PN was higher in the exposed group for canines (P = .004), premolars (P < .001), and molars (P < .001) (Table 1).

A significant association was detected between SCA and PN (Table 2). In the unadjusted analysis, which considered both strategies to evaluate the pulp condition (ie, the CTT and POD), individuals with SCA had 8.33 times more clinically intact permanent teeth with PN than the nonexposed subjects (P < .001). This association remained significant even after the adjustment for a history of orofacial trauma and folic acid use. The age variable was removed because it was not significant for models 1 and 2.

The variables sex and socioeconomic class were not selected to adjusted models because the P value was >.20. A history of orofacial trauma and folic acid use were associated with a higher occurrence of PN in both models. A lower frequency of PN was observed among adults compared with adolescents (P = .008); after adjustment, this association did not remain statistically significant (Table 2).

Discussion

The null hypothesis of this study was rejected (ie, a significant association was found between SCA and PN regardless of the diagnostic method used, Table 2). Clinically intact asymptomatic teeth with PN in SCA have been widely reported as a possible clinical complication of the disease (9–12). In this study, 17 individuals were identified with at least 1 healthy tooth with PN, 11 of whom had SCA.

Only 1 published study (12) suggests that there is an association between SCA and PN of clinically intact teeth without other associated etiologic factors. However, this study does not present an estimate of the association or testing of a hypothesis. In the unadjusted analysis of the current study, it was found that the occurrence of PN was 8.33 times higher in individuals with SCA than those without the disease (P < .001) regardless of the method of diagnosis used. This association increased after the adjustment for a history of orofacial trauma and folic acid use in both models (model 1: RR = 16.97; 95% CI, 6.80–42.37; model 2: RR = 16.14; 95% CI, 6.49–40.15). The biological plausibility for this association is possibly caused by vasoocclusive crises in people with SCA; this may lead to ischemic necrosis of the pulp tissue (3) even without other risk factors. We recognize the possibility of bias related to subjects' failure to remember a history of orofacial trauma. Orofacial injuries are serious complications for individuals with SCA because they may cause acute infections and are considered triggers in sickling crises (16, 17). These infections may evolve into osteomyelitis because of hypovascularization of the bone marrow secondary to thrombosis (9–12, 16). Therefore, considering that memory bias is characteristic of retrospective studies (18), this information was confirmed in the dental records of patients.

A comparison of the 2 groups for the variables ethnicity, sex, age, socioeconomic class, and a history of orofacial trauma showed that they were statistically similar (Fig. 1) even though the 2 groups had not been matched for these variables. The homogeneity of the groups and the adjusted multivariate analysis reduce the possibility of confounding bias. Also, because individuals of the nonexposed group were relatives of the patients with SCA, both groups came from the same population. This minimizes selection bias.

A similar prevalence of blacks and whites was observed between groups (Fig. 1). This result is not in accordance with some studies that point to a higher prevalence of SCA in black people (19). SCA is peculiar to blacks because the abnormal gene responsible for the synthesis of HbS probably originated in the central-west regions of Africa, India, and East Asia (ie, in predominantly black populations) (19, 20). A possible explanation for this divergence is that the

TABLE 1. Mean (± standard deviation) of the Number of Excluded, Vital, and Necrotic Teeth in the Exposed and Nonexposed Groups

		Exposed group			Nonexposed group		
	Total	Excluded	Vital pulp	Necrotic	Excluded	Vital pulp	Necrotic
Incisive	1356	5.80 (2.38)	2.11 (2.30)	.09 (.37)	6.65 (1.92)†	1.30 (1.91)†	.05 (.32)
Canine	4068	1.99 (1.30)	1.90 (1.26)	.11 (.56)	2.43 (1.19)*	1.57 (1.19)*	0 (.00)*
Premolar	2712	4.22 (2.84)	3.59 (2.57)	.18 (.65)	5.30 (2.71)*	2.69 (2.71)*	0 (.00)†
Molar	2712	10.29 (1.57)	1.65 (1.52)	.06 (.24)	10.83 (1.43)†	2.16 (2.43)*	0 (.00)†

*P < .05, Mann-Whitney U test.

†P < .001, Mann-Whitney U test.

TABLE 2. Socioeconomic, Demographic, and Health Variables Associated with PN

Variables	Nonexposed *n*	Exposed *n*	Unadjusted model RR (95% CI)	Unadjusted model *P* value	Adjusted model 1 (CTT) RR (95% CI)	Adjusted model 1 (CTT) *P* value	Adjusted model 2 (POD) RR (95% CI)	Adjusted model 2 (POD) *P* value
PN								
No	220	102	Ref		Ref		Ref	
Yes	6	11	8.33 (4.44–15.65)	<.001	16.97 (6.80–42.37)	<.001	16.14 (6.49–40.15)	<.001
Orofacial trauma								
No	220	110	Ref		Ref		Ref	
Yes	6	3	20.17 (11.99–33.93)	<.001	20.72 (11.88–33.14)	<.001	17.59 (9.98–30.98)	<.001
Folic acid								
No	226	14	Ref		Ref		Ref	
Yes	0	99	5.09 (2.99–8.67)	<.001	0.40 (0.18–0.90)	.027	0.43 (0.19–0.95)	.037
Sex								
Male	97	57	Ref		—	—	—	—
Female	129	56	0.78 (0.47–1.28)	.329	—	—	—	—
Age								
Adolescent	17	13	Ref		Ref		Ref	
Adult	209	100	0.96 (0.93–0.99)	.008	0.58 (0.31–1.10)	.095	0.56 (0.29–1.05)	.073
Socioeconomic class								
A/B	75	28	Ref		—	—	—	—
C	131	72	1.33 (0.75–2.37)	.329	—	—	—	—
D/E	20	13	0.78 (0.26–2.33)	.657	—	—	—	—

Ref, reference category; —, variables that were not included in the adjusted models ($P > .20$).

nonexposed group was formed by relatives of the members of the exposed group, so they have similar characteristics. Additionally, there was no difference between sex in the present investigation, which is in agreement with the observation that the gene responsible for SCA is not linked to sex but rather to autosomal inheritance (20).

The occurrence of PN was more frequent in adolescents than in adults. However, this result might be a reflection of the increased number of teeth that are decayed, lost, or filled with age. Thus, because only healthy teeth in the mouth at the time of the examination were evaluated, cases of previously treated PN in adults were not identified in the study. However, the severity and type of vasoocclusive crises varies according to age. Babies have more infections and pain with swelling in the hands and feet. In older children, pain is concentrated in the legs, arms, and belly. In adults, the most frequent crises are pain of the bones and complications caused by the damage of major organs that had occurred throughout their lives such as lesions of the liver, lungs, heart, and kidneys. In adulthood, the appearance of leg ulcers is also common (21).

The daily use of folic acid had an ambiguous effect in this study. In the univariate analysis, an increased occurrence of PN was observed (RR = 5.09; 95% CI, 2.99–8.67). However, in the multivariate models, folic acid seems to be a potential protective factor for PN (Table 2). This can be explained by the correction of the RR by confounding factors, especially after the inclusion of the variable SCA in the multivariate statistical models.

This reinforces the importance of the adoption of preventive measures against the adverse effects of SCA because there is no cure for the sickling of red blood cells. Folic acid has been proven to play a fundamental role in the speed of production and maturation of red blood cells (22), which justifies its use.

In this study, we used 2 tests to diagnose pulp vitality. According to Chen and Abbott (23), there are significant differences among dental pulp tests in terms of accuracy, reliability, and reproducibility. They compared 5 different dental pulp tests in 20 healthy patients and observed that the electric pulp (0.98) and CO_2 (0.97) tests had the highest accuracy followed closely by the laser Doppler flowmetry (0.95) and dichlorofluoromethane gas (0.91) tests. The ice test (0.85) had the lowest overall accuracy. However, refrigerant sprays have been proven to be effective and even superior to ice and ethyl chloride. A major shortcoming with these tests is that they only indirectly provide an indication of the state of the pulp by measuring a neural response rather than the blood supply, so both false-positive and false-negative results can occur (24). Cold tests are known to elicit false-negative responses, especially in elderly people because of the amount of thermal insulation provided by secondary dentin (25). In these cases, the lack of pulp sensitivity is often associated with advanced PN.

Therefore, the interpretation of data obtained from tests for pulp sensitivity is subject to variations such as the sensitivity threshold of the patient and interpretation of the response by the professional (13). Thus, the objectivity of the POD method presents advantages over other methods that are based on sensory nervous responses (13). However, despite the advantages of POD over other subjective methods for determining pulp vitality, factors related to head movement and the swallowing reflexes of the patient, probe-tooth fit, and ambient light interfere with the quality of the signal obtained (14, 26).

In the present study, 2 incisors had PN according to POD but not according to the CTT. All teeth that presented PN according to the CTT also showed no sensitivity according to POD. These data suggest a higher sensitivity of POD compared with the CTT. Despite the possibility of a false-negative, especially in thermal testing, we believe that such bias (if it occurred) did not differentiate between the groups because they were similar including in terms of age (P = .224). Thus, there were no major problems in the estimates of association.

In conclusion, SCA is a potential risk factor for PN of clinically intact teeth. The results of this study suggest that the evaluation of the condition of the pulp in clinically intact teeth should be routine in patients with SCA.

Acknowledgments

The authors deny any conflicts of interest related to this study.

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APÊNDICE B

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Dados de identificação

Título da pesquisa: **ASSOCIAÇÃO DOS PARÂMETROS CLÍNICOS E TOMOGRÁFICOS AO PERFIL MICROBIOLÓGICO DE DENTES COM NECROSE PELPAR EM PESSOAS COM ANEMIA FALCIFORME**

Pesquisadoras responsáveis: **SORAIA DE FÁTIMA CARVALHO SOUZA e CYRENE PIAZERA SILVA COSTA**

Instituição a que pertence as pesquisadoras responsáveis: **UNIVERSIDADE FEDERAL DO MARANHÃO (UFMA)**

Você está sendo convidado(a) para participar, como voluntário(a) da Pesquisa **ASSOCIAÇÃO DOS PARÂMETROS CLÍNICOS E TOMOGRÁFICOS AO PERFIL MICROBIOLÓGICO DE DENTES COM NECROSE PELPAR EM PESSOAS COM ANEMIA FALCIFORME**. Após ser esclarecido(a) sobre as informações a seguir, no caso de querer participar do estudo, assine ao final deste documento, que está em duas vias. Uma delas é a sua e a outra das pesquisadoras responsáveis. Em caso de recusa você não será penalizado(a) de forma alguma.

<u>INFORMAÇÕES DA PESQUISA</u>

OBJETIVO: Avaliar a associação dos parâmetros clínicos e radiográficos ao perfil microbiológico de dentes sem cárie e/ou obturação com morte do nervo do dente em pessoas com anemia falciforme.

PROCEDIMENTOS: Serão coletados os seus dados pessoais e será avaliada a condição do canal do dente por meio de exames clínicos pela quantidade de oxigênio e radiográficos. Aqueles dentes com morte do nervo serão tratados.

DESCONFORTOS E RISCOS AOS EXAMINADOS: É possível que ocorra leve dor no momento do exame do canal do dente e desconforto na adaptação do filme radiográfico, como também desconforto durante o tratamento do canal.

BENEFÍCIOS E RETORNO PARA OS EXAMINADOS: Os pacientes com doenças no canal do dente serão tratados pelas pesquisadoras responsáveis.

INFORMAÇÕES ADICIONAIS: Você nunca será identificado, o que preservará a privacidade de seus dados envolvidos na pesquisa. Você poderá receber esclarecimentos durante a pesquisa.

SUA PARTICIPAÇÃO NÃO É OBRIGATÓRIA, NÃO TERÁ CUSTOS E NÃO TERÁ RECOMPENSA FINANCEIRA E O SR(a) PODE DESISTIR DE PARTICIPAR A QUALQUER MOMENTO SEM QUE ISS LHE TRAGA QUALQUER PREJUÍZO DE ATENDIMENTO NA CLÍNICA DE ODONTOPEDIATRIA DA UFMA.

Esta pesquisa será avaliada pelo Comitê de Ética em Pesquisa da UFMA e se você tiver alguma reclamação, poderá procurar a coordenação desse comitê que fica na sala 07, bloco C, CEB Velho, Campus Universitário do Bacanga da UFMA, telefone (98)3301-8708.

Pesquisadoras responsáveis

SORAIA DE FÁTIMA CARVALHO SOUZA
TELEFONE:(98)8125-5554

CYRENE PIAZERA SILVA COSTA
TELEFONE:(98)8162-3333

Eu,_____________________________________, CPF n°_________________________, RG nº _____________, declaro ter sido informado e que entendi os objetivos, riscos e benefícios de participação do mesmo. Assim, concordo em participar, como voluntário, na pesquisa acima descrita e confirmo que recebi uma cópia desse documento assinado pelas pesquisadoras responsáveis.

Assinatura / impressão dactiloscópica do responsável participante:

____

São Luís, __/___/___

APÊNDICE C

FICHA CLÍNICA nº___/ Data de preenchimento:___/___/2010

Nome: ____

ANAMNESE

Outras condições sistêmicas:

☐HAS
☐Doença aterosclerótica
☐Distúrbio(s) circulatório(s)/Qual(is)? ____
☐Distúrbio(s) hormonai(s)/Qual(is)? ____
☐Distúrbio(s) metabólico(s)/Qual(is)? ____

Medicações: ____

Já sofreu algum trauma buco-dental? ( )sim ( )não /Se sim, qual dente sofreu o trauma? ____________________

EXAMES OBJETIVOS DA CONDIÇÃO PULPAR

Teste térmico a frio

Nº do dente	Resposta	
	Positiva	Negativa

Oximetria de pulso

Nº do dente	% Saturação de O_2

% Saturação de O_2 dedo: __________

APÊNDICE D

Ficha Clínica N°___ Data de Preenchimento:___/___/_____

DADOS PESSOAIS

Nome:____

Cor: ( )Preta ( )Parda ( )Branca ( ) Amarela ( ) Indígena ( ) Não sabe   Sexo: ( )Masc. ( ) Fem.

Data de nascimento: ___/___/_____ Idade:___anos

Cidade/UF:____   Telefone:_____________

DENTE:___

Análise da dor

Dor: 0 1 2 3 4 5 6 7 8 9 0   Estímulo: ( )P ( )E   Localização: ( )L ( )D ( )R   Frequência: ( )C ( )I

Intensidade: ( )A ( )L ( )P   Duração: ( )H ( )M ( )S   Exacerba por:_____________________________

Inspeção extra-oral:____

Inspeção intra-oral:____

Oximetria

D1-___ D2-___ D3-___ DM-___   P1-___ P2-___ P3-___ PM-___

Teste de vitalidade e condição periapical

Frio: ( )N ( )E ( )A   Declínio: ( )L ( )R   PV:___   PH:___   PAC:___

Exame imaginológico

Câmara pulpar: ( )normal ( )calcificada ( )nódulos ( )deformação do assoalho

Pericemento: ( )normal ( )espessado ( )hipercementose

Canal radicular: ( )amplos ( )atresiados ( )calcificados ( )reabsorção interna ( )dilaceração

( )reabsorção externa

Periápice: ( )rarefação óssea difusa ( )rarefação óssea circunscrita ( )osteíte condensante

Diagnóstico provável

( )PI ( )NP ( )PAA ( )PAC ( )AAA ( )AAC

ANEXO A

Universidade Federal do Maranhão
Pró-Reitoria de Pesquisa e Pós-Graduação
Comitê de Ética em Pesquisa

PARECER CONSUBSTANCIADO

X	PROJETO DE PESQUISA	Número do Protocolo	23115 004993/2010-71
	PROJETO DE INICIAÇÃO CIENTÍFICA	Data de entrada no CEP	22/04/2010
	TRABALHO DE CONCLUSÃO DE CURSO	Data da assembléia	03/05/2010

I - Identificação:

Título do projeto:	**Associação entre a Anemia Falciforme e necrose pulpar utilizando a oximetria de pulso e o teste de sensibilidade a frio**				
Identificação do Pesquisador Responsável:	**Profa. Dra. Soraia de Fátima C. Souza**				
Identificação da Equipe executora:	**Cyrene Piazera Silva Costa, Soraia de Fátima C. Souza e Érika B. A. F. Thomaz**				
Instituição onde será realizado:	**Universidade Federal do Maranhão (UFMA) e Setor de Odontologia do HEMOMAR**				
Área temática:	III	Multicêntrico:	Não	Data de recebimento:	27/04/2010
Cooperação estrangeira:	Não	Patrocinador:	Não	Data de devolução:	03/05/2010

II - Objetivos:

Geral:

Estimar a associação entre Anemia Falciforme e necrose pulpar, para validação da oximetria de pulso no diagnóstico de necrose pulpar a pacientes falcêmicos.

Específicos:

1. Identificação da prevalência de necrose pulpar em pacientes com Anemia Falciforme, bem como se essa anemia é um fator de risco para ocorrência de necrose pulpar.
2. Avaliação dos valores preditivos positivos da oximetria de pulso e do teste de sensibilidade frio, no diagnóstico de necrose pulpar em pacientes com Anemia Falciforme, ao mesmo tempo da análise da concordância entre a oximetria e o teste de sensibilidade a frio, no diagnóstico de necrose pulpar.

III - Sumário do projeto:

Uma das complicações bucais mais comuns da Anemia Falciforme é a necrose pulpar, geralmente assintomática e que pode acometer dentes hígidos, devido a um bloqueio do suprimento sanguíneo que nutre a polpa dental. O seu diagnóstico deve, pois, basear-se em técnicas de avaliação hemodinâmica do órgão pulpar e não apenas nos achados dos testes térmicos e mecânicos. O projeto é oriundo do programa de Pós-graduação em odontologia da Universidade Federal do maranhão. Os dados serão coletados no setor de odontologia Hemomar

IV - Comentários do relator:

O projeto de pesquisa tem relevância científica social. Do ponto de vista formal está organizado com o que é exigido. Quanto aos aspectos científicos tomando por base a Res. CNS 196/96 temos a considerar o seguinte: está ausente o CD com cópia digitalizada do projeto de pesquisa, no cronograma de atividade do projeto não consta a informação sobre o ano ou anos bem como o referido cronograma sugere pesquisa já iniciada, não claro se a pesquisa terá com objetivo monografia, dissertação. O TCLE não contém espaços para assinatura do pesquisador responsável e está com o número do CEP/UFMA incorreto

V - Pendências:

1. Apresentar cópia digitalizada do projeto de pesquisa;
2. Apresentar cronograma de atividade do projeto de pesquisa em meses e anos;
3. Declaração do pesquisador responsável sobre em que fase se encontra a pesquisa
4. Definir do que se trata o projeto (monografia, dissertação de mestrado, projeto de pesquisa etc.)
5. Apresenta no TCLE o número de contato correto deste CEP e espaço para assinatura do pesquisador responsável.

VI - Recomendações:

VII - Parecer Consubstanciado do CEP:

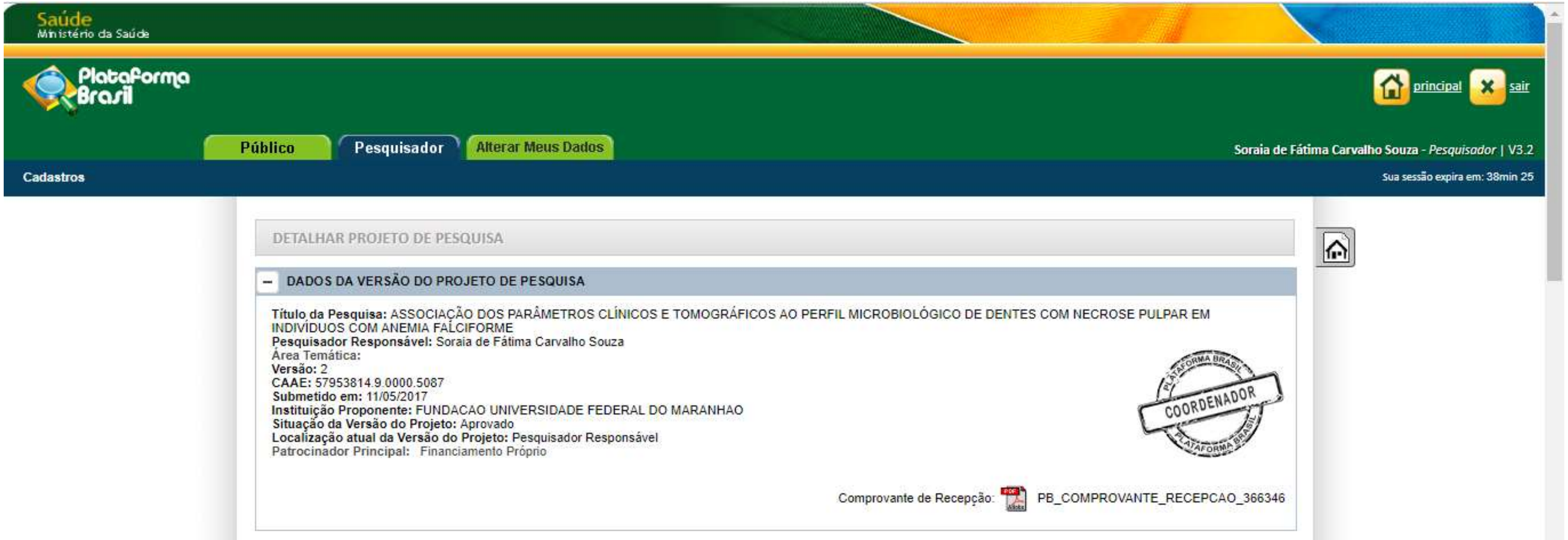
Deste modo, de acordo com relato acima exposto, o **Protocolo 23115 004993/2010-71**, referente a **Pesquisa** sob o título "**Associação entre a Anemia Falciforme e necrose pulpar utilizando a oximetria de pulso e o teste de sensibilidade a frio**" é considerado por este **CEP** como **COM PENDÊNCIA**.

VIII - Data da reunião do CEP: 03/05/2010

Helder M. Pessoa

Prof. Ms. Helder Machado pessoa
Coordenador
Comitê de Ética em Pesquisa da UFMA

ANEXO B



Saúde
Ministério da Saúde

Plataforma Brasil

principal sair

Público | Pesquisador | Alterar Meus Dados

Soraia de Fátima Carvalho Souza - *Pesquisador* | V3.2

Cadastros

Sua sessão expira em: 38min 25

DETALHAR PROJETO DE PESQUISA

— DADOS DA VERSÃO DO PROJETO DE PESQUISA

Título da Pesquisa: ASSOCIAÇÃO DOS PARÂMETROS CLÍNICOS E TOMOGRÁFICOS AO PERFIL MICROBIOLÓGICO DE DENTES COM NECROSE PULPAR EM INDIVÍDUOS COM ANEMIA FALCIFORME
Pesquisador Responsável: Soraia de Fátima Carvalho Souza
Área Temática:
Versão: 2
CAAE: 57953814.9.0000.5087
Submetido em: 11/05/2017
Instituição Proponente: FUNDACAO UNIVERSIDADE FEDERAL DO MARANHAO
Situação da Versão do Projeto: Aprovado
Localização atual da Versão do Projeto: Pesquisador Responsável
Patrocinador Principal: Financiamento Próprio

PLATAFORMA BRASIL COORDENADOR PLATAFORMA BRASIL

Comprovante de Recepção: PB_COMPROVANTE_RECEPCAO_366346

ANEXO C

The *Journal of Dental Research (JDR)* adheres to the CSE (8th Edition) editorial style. All submitted manuscripts should be formatted in this style

The *Journal of Dental Research* (*JDR*) is a peer-reviewed scientific journal dedicated to the dissemination of new knowledge and information on all science relevant to dentistry and to the oral cavity and associated structures in health and disease. The *Journal of Dental Research*'s primary readership consists of oral, dental and craniofacial researchers, clinical scientists, hard-tissue scientists, dentists, dental educators, and oral and dental policy-makers. The *Journal* is published monthly, allowing for frequent dissemination of its leading content. The *Journal of Dental Research* also offers OnlineFirst, by which forthcoming articles are published online before they are scheduled to appear in print.

Authors of all types of articles should be aware of the following guidelines when submitting to JDR.

ONLINE SUBMISSION

Submissions to the *Journal of Dental Research* are only accepted for consideration via the SAGETrack online manuscript submission site at http://mc.manuscriptcentral.com/jdr. Authors who do not have an active account within the system are required to create a new account by clicking, "Create Account," on the log-in page. The system will prompt the authors through a step by step process to create their account. Once created authors can submit their manuscripts by entering their "Author Center" and clicking the button by "Click Here to Submit a New Manuscript."

If any difficulty is encountered at any time during the account creation or submission process, authors are encouraged to contact the *Journal of Dental Research* at jdr@iadr.org.

MANUSCRIPT REQUIREMENTS BY TYPE

The *Journal of Dental Research* accepts the following types of manuscripts for consideration:

Original Research Reports: These manuscripts are based on clinical, biological, and biomaterials and bioengineering subject matter. Manuscripts submitted as research reports have a limit of 3,200 words (including introduction, materials, methods results, discussion and; excluding abstracts, acknowledgments, figure legends and references); a total of 5 figures or tables; 40 references; and must contain a 300 word abstract.

Letters to the Editor*: Letters must include evidence to support a position about the scientific or editorial content of the *JDR*. Manuscripts submitted as a letter to editor have a limit of 250 words. No figures or tables are permitted. Letters on published articles must be submitted within 3 months of the article's print publication date.

Guest Editorials*: A clear and substantiated position on issues of interest to the readership community can be considered for this manuscript type. Guest Editorials are limited to 1,000 words. No figures or tables are permitted.

Discovery!: Essays that explore seminal events and creative advances in the development of dental research are considered for the "Discovery!" section of the

journal. Manuscripts submitted for "Discovery!" have a limit of 2,500 words and a total of 2 figures or tables. Manuscripts are to be submitted by invitation only.

Critical Reviews in Oral Biology & Medicine: These manuscripts should summarize information that is well known and emphasize recent developments over the last three years with a prominent focus on critical issues and concepts that add a sense of excitement to the topic being discussed. Manuscripts are to be submitted by invitation only. Authors interested in submitting to this section must contact the Editor of *Critical Reviews in Oral Biology & Medicine,* Dr. Dana Graves, at dgraves@iadr.org for submission approval and instructions. Manuscripts submitted as Critical Reviews have a limit of 4,000 words; a total of 6 figures or tables; 60 references; and must contain a 300 word abstract.

Additional Instructions for Critical Reviews:

-It is important to include several illustrations or diagrams to enhance clarity.

Manuscripts that lack figures or diagrams typically receive a low priority score.

-Summarize important concepts in tables or flow charts or show critical data in the form of figures. NOTE: authors will need to obtain permission to reproduce a previously published figure or table.

-Due to the broad readership, abbreviations commonly recognized in one field may not be readily apparent to those in a different field. Keep abbreviation use to a minimum.

-The cover page, abstract, text, summary, figure legends, and tables should be combined into a single Word document. Figures should be submitted as a separate document.

-To view examples of recent Critical Reviews in the Journal, please click the following links:
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3318079/

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3327727/

***Brief responses to Letters to the Editor or Guest Editorials will be solicited for concurrent publication.**

Clinical Reviews (formerly Concise Reviews): These manuscripts are generally systematic reviews of topics of high clinical relevance to oral, dental and craniofacial research. Meta-analyses should be considered only when sufficient numbers of studies are available. Manuscripts that include investigations of limited study quality of under-studied areas are typically not acceptable as topics for a clinical review. Although some systematic reviews may be well done, those that receive highest scientific priority will only be considered given the very limited space allowed for these reviews in the journal.

Manuscripts submitted as Clinical Reviews have a strict limit of 4,000 words (including introduction, materials, methods results, discussion and; excluding abstracts , acknowledgments, figure legends and references); a total of 6 figures or tables; up to a maximum of 60 references; and must contain a 300 word abstract. Manuscripts above the 4,000 word/6 figure or table limit may use supplemental appendices for other supporting information that would be available online only.

Additional Instructions for Clinical

Reviews:

-It is important to include illustrations or diagrams to enhance clarity.

Manuscripts that lack figures or diagrams typically receive a low priority score.

-Summarize important concepts in tables or flow charts or show critical data in the form of figures. NOTE: authors will need to obtain permission to reproduce a previously published figure or table.

-Due to the broad readership, abbreviations commonly recognized in one field may not be readily apparent to those in a different field. Keep abbreviation use to a minimum.

-The cover page, abstract, text, summary, figure legends, and table(s) should be combined into a single Word document. Figures should be submitted as a separate document.

-To view examples of recent Clinical Reviews in the Journal, please click the following links: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5613886/ or https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5004242/

All submissions must include a title page and be accompanied by a cover letter and list of suggested reviewers. Cover letters should certify the research is original, not under publication consideration elsewhere, and free of conflict of interest. Title pages should include: abstract word count, total word count (Abstract to Acknowledgments), total number of tables/figures, number of references, and a minimum of 6 keywords. Keywords cannot be words that have been included in the manuscript title. Key words should be selected from Medical Subject Headings (MeSH) to be used for indexing of articles. See: http://www.nlm.nih.gov/mesh/MBrowser.html for information on the selection of key words.

Please submit the names and email addresses of four preferred reviewers when prompted by the SAGETrack system. Preferred reviewers cannot be colleagues at the contributors' institution or present or former collaborators.

TITLES

Titles can consist of a maximum of 75 characters (including spaces) . Titles do not normally include numbers, acronyms, abbreviations or punctuation. The title should include sufficient detail for indexing purposes but be general enough for readers outside the field to appreciate what the paper is about.

ACKNOWLEDGMENTS

Authors are required to report all sources of support for their project or study, including but not limited to: grant funds, commercial sources, funds from a contributors' institution. Do not refer to a study being "partially funded by the cited sources." Consultancies and funds paid directly to investigators must also be listed. Authors are required to specify during the submission process if their paper received funding from NIH, NIDCR, or any other NIH Institute or Center and provide the grant number. To comply with the NIH Public Access Mandate, for qualifying NIH- funded papers, the *Journal of Dental Research* will deposit the final, copyedited paper to PubMed Central on behalf of the authors.

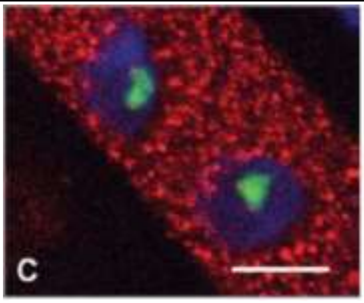
Any perceived or actual conflicts of interest need to be identified in the acknowledgments section. The *JDR* abides by the International Committee of Medical Journal Editors guidelines for the Ethical Considerations in the Conduct and Report of Research (http://www.icmje.org). Authors are requested to include this information in the acknowledgments section and the corresponding author must confirm that all co-authors have reported any potential conflicts.

FIGURE AND TABLE REQUIREMENTS

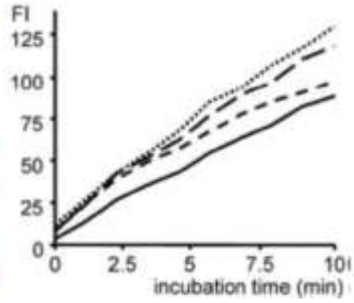
These guidelines are intended to aid authors in providing figures that will reproduce well in both print and online media. Submitting digital image files that conform to these guidelines will prevent delays in the review and publication processes, and maximize the published quality of your figures.

Figure Types

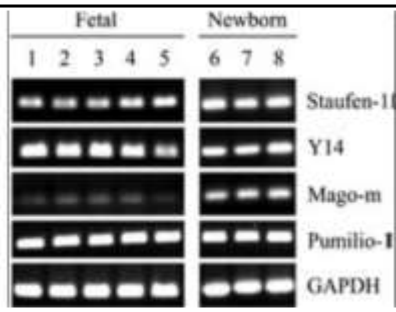
JDR figures can fall into one of three categories: **Continuous-tone images**, **Line- art images**, and **Combination images.** Each image type has specific requirements in terms of the resolution needed for publication and the file types best suited for the figure. See the following panels for examples and requirements.



Continuous-tone Image
Minimum resolution: 300dpi.
Preferred File Formats: TIFF, Bitmap.



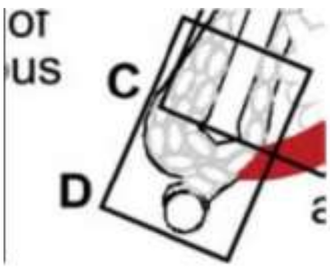
Line-art Image
Minimum resolution: 800dpi.
Preferred File Formats: EPS, PowerPoint, Illustrator.



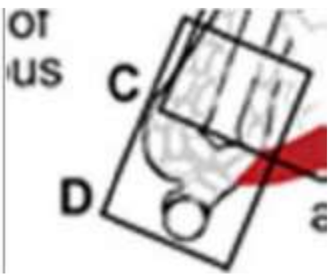
Combination Image
Minimum resolution: 800dpi.
Preferred File Formats: PDF, EPS,
PowerPoint, Illustrator, InDesign.

Resolution

In order for a figure to be used in publication, its Digital Image File must have the required resolution when it is created. The resolution cannot be raised *after* the original image is made. Attempting to do so (for example, with Adobe Photoshop's© *"Image Size"* command) results in the addition of artificial pixels that distort the image and lower its sharpness. The figures on the right show an example of this reduced sharpness.



Line-art supplied at high resolution (1000dpi).



Using "Image Size" to go from 300 DPI to 1000 DPI.

Image Integrity Guidelines

The International Committee of Medical Journal Editors (ICMJE) recommendations note that scientific misconduct includes deceptive manipulation of images. Figures submitted to the *Journal of Dental Research* should be minimally processed and should reflect the integrity of the original data in the image(s). Adjustments to images in brightness, contrast, or color balance should be applied equally to the entire image, provided they do not distort any data in the figure, including the background. Selective adjustments and touch-up tools used on portions of a figure are not appropriate. Images should not be layered or combined into a single image unless it is stated that the figure is a product of time-averaged data. All adjustments to image date should be clearly disclosed in the figure legend. Images may be additionally screened to confirm faithfulness to the original data. Authors are expected to supply raw image data upon request. Authors should also list tools and software used to collect image data and should document settings and manipulations in the Methods section.

These guidelines were derived from those provided by the *Journal of Cell Biology* and *Nature*:

http://jcb.rupress.org/editorial-policies#data-integrity

https://www.nature.com/authors/policies/image.html

Fonts

Limit fonts used in any figure to Times, Times New Roman, Arial, Frutiger, and Sabon.

Other fonts cannot be guaranteed to reproduce properly.

Files containing figures and tables should be clearly labeled to indicate their placement in the text or appendix. Tables should be viewable in a portrait view. Tables that are created in a landscape view are more suitable for an appendix.

If the online version is in color and the printed version in black and white, please submit separate files for each version. Figures should be identical except in color or grayscale. The cost of color figures in the print version will be borne by the authors. Rates for color reproduction are $300 per initial page of color and $150 for each additional page of color. However, there are no charges for figures and diagrams printed in black and white. Color figures many be included

in the online version of *JDR* with no extra charges.

REFERENCES

The *Journal of Dental Research (JDR)* adheres to the CSE (8th Edition) editorial style. All submitted manuscripts should be formatted in this style: http://www.scientificstyleandformat.org/Tools/SSF-Citation-Quick-Guide.html.

SUPPLEMENTAL FILES

Additional supporting data may be referenced as a supplemental appendix for publication online only. All supplemental appendix files must be submitted with the manuscript for review. Supplementary files will be subjected to peer-review alongside the article.

Supplementary files will be uploaded as supplied. They will not be checked for accuracy, copyedited, typeset or proofread. The responsibility for scientific accuracy and file functionality remains with the authors. A disclaimer will be displayed to this effect with any supplementary material published. Supplemental files may include additional figures or tables that exceed the Journal's limit. Material intended for the supplemental appendix must have "supplemental" or "appendix" in the file name upon upload. When formatting your supplemental files, please follow these instructions:

> Authors should provide a single Word file with all Appendix content. Figures and tables should be included in the main Appendix file so they can appear immediately alongside their captions. High resolution figures may also be supplied separately if authors wish, but they also must be copied into the Word file so everything can be kept together.

Be sure to run spell check and proofread the text.

Remove all highlighting/other colors. Use one font throughout.

> The Appendix should include the title of the article and all authors. Page numbers are recommended.

Figures and Tables should be labeled Appendix Figure/Table 1, Appendix Figure/Table 2, etc. Avoid labeling as S1, S2, and so forth.

All table footnotes and figure legends should be included.

Preferably, authors shouldn't label separate parts as "Appendix 1", "Appendix 2", etc.; just use section heads as in a regular article.

Language Editing: Manuscripts submitted for publication consideration should be written in English. Prior to submission, if a manuscript would benefit from professional editing, authors may consider using a language-editing service. Suggestions for this type of service can be found at www.iadr.org/EditingServices. The *Journal of Dental Research* does not take responsibility for, or endorse these services, and their use has no bearing on acceptance of a manuscript for publication.

GENERAL INFORMATION FOR AUTHORS SUBMITTING A MANUSCRIPT PRIOR PUBLICATION

Manuscripts submitted to the *Journal of Dental Research* are accepted for consideration giving the understanding that it contains original material that has not been submitted for publication or has been previously published elsewhere. Any form of publication other than an abstract only constitutes prior publication.

ICMJE COMPLIANCE STATEMENT

Manuscript submission guidelines for the *Journal of Dental Research* follow the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" set forth by the International

Committee of Medical Journal Editors (ICMJE). For additional information please visit the ICMJE web site at http://www.icmje.org/.

CONSORT 2010 CHECKLIST COMPLETION RANDOMIZED CLINICAL TRIALS POLICY

Manuscripts reporting a randomized clinical trial are required to follow the CONSORT guidelines. The Journal requires authors of pre -clinical animal studies submit with their manuscript the Animal Research: Reporting In Vivo Experiments (ARRIVE) guidelines. Authors of human observations studies in epidemiology are required to review and submit a STROBE statement. When uploaded to the SAGETrack system, any checklists completed by authors should be given

a supplementary file designation. Authors who have completed the ARRIVE guidelines or STROBE checklist should include as the last sentence in the Methods section a sentence stating compliance with the appropriate guidelines/checklist.

Additional guidance on compliance with various research guidelines can be found on the Guideline Information - Enhancing the Quality and Transparency of Health Research: www.equator-network.org.

The CONSORT checklist can be downloaded from:

http://www.equator-network.org/reporting-guidelines/consort/

The ARRIVE guidelines can be found here:

www.nc3rs.org.uk/downloaddoc.asp?id=1206&page=1357&skin=0

The STROBE checklists can be found here:

www.strobe-statement.org/index.php?id=strobe-home

The *Journal of Dental Research* requires authors to register their clinical trials in a public trials registry. Authors of manuscripts describing such studies are asked to submit the name of the registry and the study registration number prior to publication. Authors are asked to include their clinical trial registration number at the end of their abstracts. In accordance with the aforementioned "Uniform Requirements for Manuscripts Submitted to Biomedical Journals," clinical trials will only be considered for publication if they are registered.

INSTITUTIONAL REVIEW BOARD AND WRITTEN INFORMED CONSENT

For protocols involving the use of human subjects, authors should indicate in their Methods section that subjects' rights have been protected by an appropriate Institutional Review Board and written informed consent was granted from all subjects. When laboratory animals are used, indicate the level of institutional review and assurance that the protocol ensured humane practices.

PUBLIC GENE DATA

Prior to submission, the *Journal of Dental Research* asks that novel gene sequences be deposited in a public database and the accession number provided to the Journal. Authors may want to use the following Journal approved databases:

GenBank: www.ncbi.nlm.nih.gov/Genbank/submit.html

EMBL: www.ebi.ac.uk/embl/Submission/index.html

DDBJ: www.ddbj.nig.ac.jp/index-e.html

Manuscript submissions including microarray data should include the information recommended by the MIAME guidelines in their submission, and/or identify the submission details for the experiments details to one of the publicly available databases such as Array Express or GEO. Information on MIAME, Array Express and GEO can be found by clicking on the corresponding links below:

MIAME: www.mged.org/Workgroups/MIAME/miame.html

ArrayExpress: www.ebi.ac.uk/arrayexpress

GEO: www.ncbi.nlm.nih.gov/geo

FUNDING COMPLIANCE STATEMENT

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ANEXO D

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a. The paragraph is the ideal unit of organization. Paragraphs typically start with an introductory sentence that is followed by sentences that describe additional detail or examples. The last sentence of the paragraph provides conclusions and forms a transition to the next paragraph. Common problems include one-sentence paragraphs, sentences that do not develop the theme of the paragraph (see also section "c," below), or sentences with little to no transition within a paragraph.

b. Keep to the point. The subject of the sentence should support the subject of the paragraph For example, the introduction of authors' names in a sentence changes the subject and lengthens the text. In a paragraph on sodium hypochlorite, the sentence, "In 1983, Langeland et al, reported that sodium hypochlorite acts as a lubricating factor during instrumentation and helps to flush debris from the root canals" can be edited to: "Sodium hypochlorite acts as a lubricant during instrumentation and as a vehicle for flushing the generated debris (Langeland et al, 1983)." In this example, the paragraph's subject is sodium hypochlorite and sentences should focus on this subject.

c. Sentences are stronger when written in the active voice, that is, the subject performs the action. Passive sentences are identified by the use of passive verbs such as "was," "were," "could," etc. For example: "Dexamethasone was found in this study to be a factor that was associated with reduced inflammation," can be edited to: "Our results demonstrated that dexamethasone reduced inflammation." Sentences written in a direct and active voice are generally more powerful and shorter than sentences written in the passive voice.

d. Reduce verbiage. Short sentences are easier to understand. The inclusion of unnecessary words is often associated with the use of a passive voice, a lack of focus, or run-on sentences. This is not to imply that all sentences need be short or even the same length. Indeed, variation in sentence structure and length often helps to maintain reader interest. However, make all words count. A more formal way of stating this point is that the use of subordinate clauses adds variety and information when constructing a paragraph. (This section was written deliberately with sentences of varying length to illustrate this point.)

e. Use parallel construction to express related ideas. For example, the sentence, "Formerly, endodontics was taught by hand instrumentation, while now rotary instrumentation is the common method," can be edited to "Formerly, endodontics was taught using hand instrumentation; now it is commonly taught using rotary instrumentation." The use of parallel construction in sentences simply means that similar ideas are expressed in similar ways, and this helps the reader recognize that the ideas are related.

f. Keep modifying phrases close to the word that they modify. This is a common problem in complex sentences that may confuse the reader. For example, the statement, "Accordingly, when conclusions are drawn from the results of this study, caution must be used," can be edited to "Caution must be used when conclusions are drawn from the results of this study."

g. To summarize these points, effective sentences are clear and precise, and often are short, simple and focused on one key point that supports the paragraph's theme.

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ANEXO E – Produção Científica no período de 2014-2017

Original Research
Oral Diagnosis

Is sickle cell anemia a risk factor for severe dental malocclusion?

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Abstract: The aim of this study was to investigate possible associations between sickle cell anemia (SCA) and the severity of dental malocclusion (MO). This was a retrospective cohort study of 93 individuals with SCA (G1) and 186 individuals without the disease (G2). SCA patients were randomly selected by a simple draw from patients treated in the *Centro de Hematologia e Hemoterapia do Maranhão* (HEMOMAR) in northeastern Brazil. Patients aged between 16 and 60 were included after being tested for the hemoglobin S gene. G2 consisted of individuals living in the same residence as the patients. The Dental Aesthetic Index (DAI), as well as some morphological deviations not included in DAI, were used for the orthodontic evaluation of MO. Poisson regression with robust variance adjustment was employed to estimate relative risk (RR). In the multivariate analysis, SCA was associated with moderate (RR = 1.36) and very severe MO (RR = 8.0). SCA is correlated with anterior tooth loss (RR = 1.94), anterior spacing (RR = 1.66), overjet (RR = 1.87), anterior crossbite (RR = 1.94), and open bite (RR = 1.94). Thus, SCA is a risk factor for moderate and very severe MO.

Keywords: Malocclusion; Anemia, Sickle Cell; Epidemiology.

Declaration of Interests: The authors certify that they have no commercial or associative interest that represents a conflict of interest in connection with the manuscript.

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Introduction

Sickle cell anemia (SCA) is an autosomal recessive nonsex-linked hemoglobinopathy predominant among Afro-descendants. Its cause is a point mutation in the β-chain of hemoglobin which results in an abnormal hemoglobin variant called hemoglobin S (HbS).[1] SCA has been categorized as a public health issue as it affects a significant percentage of the world's population. It is considered the most prevalent inherited disease in Brazil; the state of Bahia has the highest incidence of SCA at 15.4 cases per 10,000 live births and Maranhão is in fourth position with 7.1 cases per 10,000 live births.[2]

Because red blood cells need to be constantly produced towing to their short lifespan, SCA patients commonly suffer from hyperplasia and compensatory expansion of the bone marrow, which may result in bone changes even of the craniofacial bone structures. One study reported depression of the nasal bridge, midfacial overgrowth resulting in maxillary protrusion, and the formation of a thick trabecular pattern.[3] Furthermore, a significantly more pronounced vertical pattern (clockwise) is observed as inferior and total face height angles and posterior rotation of the mandible are increased in SCA.[4]

Changes to the craniofacial bone structures are believed to be factors that contribute to the development of dental malocclusion (MO). Moreover, delayed tooth eruption and an unusually high degree of periodontitis are common in SCA, both of which can also result in MO.[4] Thus, the rationale for an association between this disease and dental MO is plausible. However, the role of SCA in the establishment of MO remains controversial as far as the different types of MO and the varying degrees of severity are concerned. Most studies on this issue have been performed with small samples, using descriptive, case report, or cross-sectional designs, and with no control for confounding factors, on samples that grouped the deciduous and mixed dentitions, and without considering the severity of MO.[5] Thus, the objective of this study was to assess the possible associations between SCA and the severity of MO. The null hypothesis tested was that there is no difference in the severity of MO between patients with SCA and control subjects.

Methodology

Design and location of study

This was a cohort study with both retrospective and prospective phases. The exposure (SCA) and the outcome of interest (MO) were already existent at the time of the study and the allocation of subjects in the study took place from retrospectively investigating for the exposure condition in hospital records. Therefore, the exposure was retrospectively assessed; however, the outcome was prospectively diagnosed. The study was conducted in the *Centro de Hematologia e Hemoterapia do Maranhão* (HEMOMAR), a state healthcare institution responsible for government policies related to hematology and transfusion medicine in the State of Maranhão, Brazil. Because this is the referral center for the treatment of hematologic diseases, it covers the entire state.

Subjects of study

SCA Group

SCA was diagnosed using the electrophoresis technique. The diagnosis of SCA by neonatal screening for the *HbS* gene was confirmed from the records. Individuals with SCA, aged between 16 and 60 years old (since the roots of the second molars have fully developed by the age of 16), were randomly selected from the patients' records of HEMOMAR to form the study group (G1). Randomization of G1 was performed by draw.

Control Group

HEMOMAR performs extended family screening for SCA, as part of a national screening program for congenital diseases. Relatives without SCA living with the individuals in G1 were recruited to form a control group (G2). Absence of the disease was confirmed by checking the medical records of the social services department of HEMOMAR. Randomization of G2 was performed by draw.

Exclusion criteria for both groups were history of orthodontic treatment (OT), endocrine-metabolic disorders, mental retardation, genetic craniofacial syndromes and disorders known to have a negative effect on occlusion, and history of head and neck surgery or head and neck radiation, as well as refusal to participate in the study.

Calculated sample size

Seventy-eight individuals with SCA and 156 without SCA would have 80% power to identify differences of 20% in the incidence of MO between the two groups [Relative Risk (RR) = 1.4] with an alpha error of 5% estimating a frequency of 50% of MO in those without SCA. Finally, 20% more patients were enrolled in the study to compensate for an expected loss to treatment; thus, giving a total of 279 subjects (93 in G1 and 186 in G2).

Data collection

Data was collected between January and June 2011. Demographic data of participants were obtained from patients' records in HEMOMAR including name, address, phone number, date of birth, gender, and self-reported race categorized as Black, White, or other according to the criteria of the *Instituto Brasileiro de Geografia e Estatística – IBGE*.[6] Information on socioeconomic status according to economic classification criteria in Brazil[7] was obtained by interviews (categorized as A/B, C, or D/E).

The Dental Aesthetic Index (DAI) was used to evaluate the severity of MO and the necessity of OT. Individuals were categorized as: without MO or with mild MO (without the need of treatment), moderate MO (elective treatment), severe MO (desirable treatment), and very severe MO (treatment essential). This index is recommended by the World Health Organization and has good reproducibility and validity. Moreover, posterior crossbite, posterior open bite, and overbite, which are not covered by DAI, were also assessed.[8]

The orthodontic clinical examinations were conducted in a dental office by three experienced examiners (minimum Kappa = 0.9) under artificial light using a community periodontal index probe and disposable wooden spatulas. Prior to the evaluation, each subject was submitted to bite deprogramming using plastic strips (Long strips).

Statistical analysis

Data were analyzed using Stata software version 10 (Stata Corp., College Station, USA). The normality of the sample was tested using the Shapiro-Wilk test. The 95% confidence intervals (CI) for severity of MO and need for OT were estimated for both groups and compared using the Kruskal-Wallis and Chi-square tests. A level of significance of 5% was adopted to reject the null hypothesis. The associations between SCA and severity of MO were estimated by separate Poisson regression models with robust adjustments of the variance; the unadjusted and adjusted relative risk (RR) and respective 95%CI were calculated. Multinomial logistic regression analysis is used when the outcome has more than two categories and odds ratio (OR) is used as an indicator of the prevalence ratio (PR) or RR. However, recent studies indicate that OR can be a biased estimator of PR and RR, often generating overestimated measures of association, and thus leading to spurious interpretations. Hence, the use of log multinomial regression models and Poisson regression with specific robust variance have been proposed.[9]

Additional models were tested considering each of the components of DAI as outcomes, as well as posterior crossbite, posterior open bite, and overbite, which are types of MO that are not included in DAI.

The criterion used for covariables to enter the model as potential confounding factors was a statistical significance of < 20% ($p < 0.20$) in the univariate analysis. The selection of covariables was by backwards stepwise regression to eliminate variables with $p > 0.10$.

Ethical aspects

The study was approved by the local Research Ethics Committee (protocol #23115-04993/2010-71). All individuals were invited to participate in the study and signed written consent forms.

Results

The characteristics of the study participants are shown in Table 1. DAI was higher in G1 than in G2 (Figure). The variables age and DAI had asymmetric distributions in both groups ($p < 0.001$). There were no statistically significant differences between groups with respect to sociodemographic variables; however, the median DAI was higher in G1 than in G2 ($p < 0.001$).

Significant differences between groups were found for ethnicity, gender, and socioeconomic status in relation to MO ($p < 0.05$). These variables were inserted into the adjusted regression models, as was age.

The frequency of normal occlusion of teeth or mild MO was higher in G2 than in G1, while very severe MO was most commonly observed in G1 ($p < 0.001$), showing that individuals with SCA have a greater need of OT than controls (Table 2).

In the unadjusted analysis, individuals in G1 had a greater risk of developing moderate to very severe MO compared to those in G2. After adjusting for gender, ethnicity, age, and socioeconomic status, significant associations remained for moderate (RR = 1.36; 95%CI: 1.07–1.73) and for very severe MO (RR = 8.0; 95%CI: 3.21–19.33) (Table 2). Adjusted Poisson models explained 4% of the variability in moderate ($R^2 = 0.04$) MO, 15% of the variability in severe MO ($R^2 = 0.15$), and 37% of the variability in very severe MO ($R^2 = 0.37$).

On evaluating the frequency of each of the DAI components, SCA was associated to an increased prevalence of anterior tooth loss (RR = 1.94; 95%CI: 1.33–2.83), anterior spacing (RR = 1.66; 95%CI:

Table 1. Sociodemographic characteristics of the participants.

Variables	G1 (n = 93)		G2 (n = 186)		p
	n	% (95%CI)	n	% (95%CI)	
Ethnicity					0.62[b]
White	3	3.2 (0.7–9.1)	8	4.3 (18.7–83.0)	
Black	61	65.6 (55.0–75.1)	111	59.7 (52.2–67.8)	
Other	29	31.2 (22.0–41.6)	67	36.0 (29.1–43.4)	
Gender					0.20[b]
Female	47	50.5 (40.0–61.1)	109	58.6 (51.2–65.8)	
Male	46	49.5 (38.9–60.0)	77	41.4 (34.2–48.8)	
Economic status[a]					0.32[b]
A–B	23	24.7 (16.4–34.8)	61	32.8 (26.1–40.0)	
C	59	63.5 (52.8–73.2)	109	58.6 (51.2–65.8)	
D–E	11	11.8 (6.0–20.2)	16	8.6; 5.0–13.6)	
		Median (IQD)		Median (IQD)	
Age		26.0 (10.0)		26.0 (9.0)	0.59[c]
DAI		30.0 (14.0)		29.5 (7.0)	<0.001[c]

SCA: Sickle Cell Anemia; 95%CI: 95% Confidence Interval; IQD: Inter-Quartile Deviation; DAI: Dental Aesthetic Index; [a]Economic status according to the *Associação Brasileira de Empresas de Pesquisa* Criteria;[7] [b]Chi-squared test; [c]Kruskal-Wallis test.

1.29–2.14), overjet (RR = 1.87; 95%CI: 1.49–2.36), anterior crossbite (RR = 1.94; 95%CI: 1.33–2.83), and open bite (RR = 1.94; 95%CI: 1.33–2.83). In addition, SCA was associated with a 25% reduction in the prevalence of irregularities of the maxilla (RR = 0.75; 95%CI: 0.65–0.86) and 12% of the mandible (RR = 0.88; 95%CI: 0.81–0.95). It was not possible to estimate any association between SCA and diastema due to the absence of diastema in control subjects (Table 3).

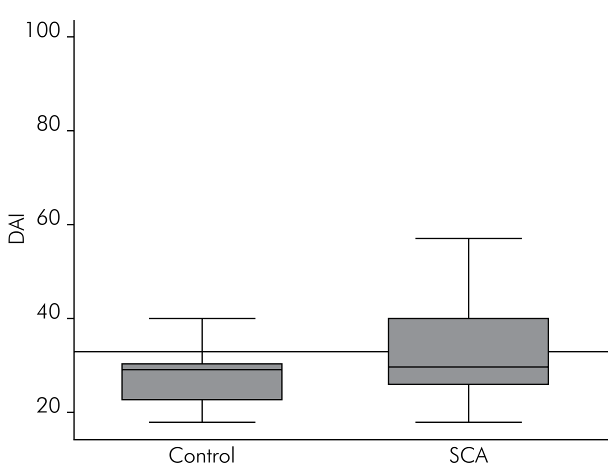

Figure. Box-plot of the dental aesthetic index distribution in sickle cell anemia and control individuals

Associations were not found between SCA and posterior open bite, posterior crossbite, or overbite (Table 4).

Discussion

The null hypothesis of this study was rejected. Significant associations were found between the SCA with moderate ($p = 0.01$) and very severe ($p < 0.001$) MO in permanent dentition. SCA patients presented a 1.36 times higher risk of developing moderate MO and 8.0 times higher risk of developing very severe MO than individuals without the disease. In particular, these associations are because us of the following DAI components: tooth loss, tooth spacing, overjet, anterior crossbite, and anterior open bite. In addition, it was observed that 30.1% of individuals with SCA had indications for mandatory OT compared to only 2.7% of the control subjects.

This result confirms the findings of Onyeaso and Costa[10] who, on investigating the relationship between dental aesthetics and the need for OT in individuals with SCA in Nigeria, found that 73.3% of the sample had severe MO with a need of OT. The authors attributed the findings to the degree of craniofacial changes in these individuals. A higher prevalence of overjet and overbite (35%) was also found among SCA patients when compared with individuals without SCA (15%) in a small case-control

Table 2. Severity of dental malocclusion and the need for orthodontic treatment.

Severity/OT	G1		G2		Unadjusted	Adjusted[a]
	n	% (95%CI)	n	% (95%CI)	RR (95%CI)	RR (95%CI)
Normal occlusion or mild MO/None	22	23.7 (15.4–36.6)	88	47.3 (39.9–54.7)	1.0	1.0
Moderate MO/Optional	36	38.7 (28.8–49.4)	72	38.7 (31.7–46.1)	1.38 (1.06–1.80)	1.36 (1.07–1.73)
Severe MO/Highly desirable	7	7.5 (3.1–14.9)	21	11.3 (7.1–16.7)	1.25 (0.59–2.66)	1.33 (0.76–2.34)
Very severe MO/Essential	28	30.1 (21.0–40.5)	5	2.7 (0.9–6.2)	10.42 (4.27–25.37)	8.0 (3.21–19.33)

MO: Dental Malocclusion; OT: Orthodontic Treatment; DAI: Dental Aesthetic Index; 95%CI: 95% Confidence Interval; RR: Risk Relative; [a]Adjusted for self-reported ethnicity, gender, age, and socioeconomic status.

Table 3. Dental malocclusion in anterior segments according to the components of the Dental Aesthetic Index in sickle cell anemia patients and control subjects (northeastern Brazil, 2011).

	G1		G2		*p*	Unadjusted	Adjusted[c]
	n	%	n	%		RR (95%CI)	RR (95%CI)
Absence of teeth					0.43[a]		
Yes	39	41.9	69	37.1		1.13 (0.83–1.53)	1.94 (1.33–2.83)
No	54	58.1	117	62.9		1.0	1.0
Crowding					0.37[a]		
Yes	74	79.6	139	74.7		1.06 (0.93–1.26)	1.06 (0.93–1.21)
No	19	20.4	47	25.3		1.0	1.0
Spacing					< 0.001[a]		
Yes	56	60.2	67	36.0		1.67 (1.30–2.15)	1.66 (1.29–2.14)
No	37	39.8	119	64.0		1.0	1.0
Incisor diastema					< 0.001[a]		
Yes	14	15.0	0	0.0			
No	79	85	186	100.0			
Irregularity of the maxilla					< 0.001[a]		
Yes	64	68.8	173	93.0		0.74 (0.64–0.85)	0.75 (0.65–0.86)
No	29	31.2	13	7.0		1.0	1.0
Irregularity in mandible					< 0.001[a]		
Yes	80	86.0	184	98.9		0.87 (0.80–0.94)	0.88 (0.81–0.95)
No	13	14.0	2	1.1		1.0	1.0
Overjet					< 0.001[a]		
Yes	63	67.7	67	36.0		1.88 (1.48–2.38)	1.87 (1.49–2.36)
No	30	32.3	119	64.0		1.0	1.0
Anterior crossbite					0.002[a]		
Yes	12	12.9	6	3.2		4 (1.55–10.34)	3.9 (1.51–10.10)
No	81	87.1	180	96.8		1.0	1.0
Open bite					< 0.001[a]		
Yes	12	12.9	1	0.5		24 (3.16–182.44)	18.46 (2.5–136.45)
No	81	87.1	185	99.5		1.0	1.0
Molar relationship					0.10[b]		
Normal	4	4.3	12	6.4		1.0	1.0
Half cusped altered	81	87.1	167	89.8		1.02 (0.96–1.09)	1.03 (0.96–1.09)
One cusped altered	8	8.6	7	3.8		1.81 (0.88–3.73)	1.57 (0.78–3.16)

RR: Relative Risk; 95%CI: 95% Confidence Interval; [a]Chi-squared test; [b]Kruskal-Wallis test; [c]Adjusted for age, gender, ethnicity, and socioeconomic status; [d]Pearson chi-squared test with robust variance.

Table 4. Malocclusion in the posterior segments (components not included in the DAI) between sickle cell anemia patients and control subjects (northeastern Brazil, 2011).

	G1		G2		P[a]	Unadjusted	Adjusted[b]
	n	%	n	%		RR (95%CI)	RR (95%CI)
Posterior open bite					0.72		
Yes	28	30.1	60	32.3		0.93 (0.64–1.36)	0.99 (0.69–1.42)
No	65	69.9	126	67.7		1.0	1.0
Posterior crossbite					0.21		
Yes	10	10.8	12	6.5		1.67 (0.75–3.72)	1.78 (0.82–3.86)
No	83	89.2	174	93.5		1.0	1.0
Overbite					0.06		
Yes	24	25.8	30	16.1		1.60 (0.99–2.57)	1.54 (0.97–2.46)
No	69	74.2	156	83.9		1.0	1.0

RR: Relative Risk; 95%CI: 95% Confidence Interval; [a]Chi-squared test; [b]Adjusted for age, gender, ethnicity, and socioeconomic status.

study comprised 37 cases and 24 controls in Nigeria.[11] Furthermore, Class II MO was found in 21% of SCA subjects compared to 2% of controls (p <0.05) in a cross-sectional study also in Nigeria.[12]

In the present study, associations were found between SCA and higher frequencies of tooth loss (RR = 1.94), spacing in the incisor segments (RR = 1.66), anterior open bite (RR = 1.94), overjet (RR = 1.87), and anterior crossbite (RR = 1.94). Although some of these findings do not have precedents in the literature, they are in agreement with other studies that suggest an association between SCA and MO is plausible. Brown and Sebes[13] and Alves *et al*.[14] reported that the increased number of MO in patients with SCA may be related to muscle imbalance, lack of lip seal or changes in the bone base, and configurations in SCA patients. However, these investigations did not report on the extent of the associations, nor did they adjust for potential confounding factors using multiple regression analyses. Moreover, some studies did not use a control group and thus were only descriptive,[13] or included different age groups[10,12] and small sample sizes,[11,13] and one was a case report.[15]

There are records that frequent vaso-occlusive crises cause ischemic necrosis in individuals with SCA.[5,14] This process can also affect the maxillomandibular blood supply, which may explain the increased tooth loss and spacing of teeth such as was observed in this study. Overjet has been highlighted in previous studies as one of the most common MO in individuals with SCA.[10,16] Taylor *et al*.[16] and Costa *et al*.[17] reported a prevalence of overjet of 48.2% and 56%, respectively, in individuals with SCA. The results of the current study are similar, with a high prevalence of overjet (67.7%), one of the most common MO. However, paradoxically, an association between SCA and anterior crossbite was also found. Most studies point to overjet and not anterior crossbite as a possible consequence of SCA.[14,16,17] One possible explanation is given by Williams *et al*.[18] who suggested that SCA may be associated with increased bone marrow activity and dysplastic maxillary growth.

This study found a prevalence of 79.6% of crowding and 60.2% of spacing between incisor segments in G1 (Table 3), thus differing from that of Costa *et al*.[17] who found a prevalence of 49% for crowding and 30.8% for spacing in the anterior segments of the upper and lower arches. These differences may partly be explained by differences in the age groups of the populations studied, as the current study enrolled an older population (16–60 years) than that in the study by Costa *et al*.[17] (10–45 years), as well as by differences in the power of the sample as the authors did not specify the parameters employed to enroll the 140 subjects that formed their sample.

One limitation of the current study is that the sample size was very small to evaluate differences in the frequency of diastema (not observed in G2). However, the sample size estimates were calculated for an alpha error of 5% and a beta error of 20%, which is conventionally considered to be acceptable. Moreover, the sample size was adequate for the main outcome of the study, namely MO as classified according to DAI. Studies with retrospective designs are limited in respect to the data that can be collected. The high range of ages can introduce some bias, as age is also associated to tooth loss. However, to control this, all associations for that variable were adjusted.

The strengths of the study include the longitudinal design, which is one of the best types to test etiological hypotheses, selection of G1 by random sampling in a state referral service, and the choice of G2 from the same community to reduce possible selection bias. Furthermore, the objective criterion to define SCA based on blood tests at birth, as well as the diagnosis of MO, according to the international gold standard with assessments carried out by experienced examiners, are methodological safeguards that minimize potential measurement biases.

Conclusions

SCA appears to be a risk factor for moderate and very severe MO, especially when related to anterior tooth loss, anterior spacing, overjet, anterior crossbite, and open bite.

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Original Article

Dental care provided to sickle cell anemia patients stratified by age: A population-based study in Northeastern Brazil

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ABSTRACT

Objective: To assess differences in the dental care provided to sickle cell anemia (SCA) patients depending on age. This retrospective study used secondary data from the dental records of the Center of Hematology and Hemotherapy in Maranhão (HEMOMAR). **Materials and Methods:** Data were obtained from 574 dental records of patients with SCA treated or under treatment in the Dental Department of HEMOMAR from 2000 to 2011. Data on the gender, age, duration of dental treatment, number of patients submitted to periodontal treatment (PT), number of filled teeth (FT), teeth extracted (EX), endodontically treated teeth (ET), and reason for the dental procedures were collected. The Kruskal–Wallis test together with Dunn's *post hoc* test, Chi-square test, and Spearman's correlation was used for statistical analysis. An alpha error of 5% was considered acceptable. **Results:** Significant differences were found for FT, EX ($P < 0.05$), ET and PT ($P < 0.001$) between the age groups. There were fewer FT in children compared to other age groups ($P < 0.001$). The most common reasons for restorations and endodontic treatment were dental caries (100%) and irreversible pulpitis (55.6%), respectively. The main reasons for teeth extractions were residual roots (21.3%), chronic apical periodontitis (19.7%), and crown destruction (19.3%). There were positive correlations between age and EX ($r = 0.93$; $P = 0.025$) and ET ($r = 0.92$; $P = 0.028$). **Conclusions:** FT, ET, EX, and PT procedures become more common in older patients. Tooth decay is the main reason for dental treatment in SCA patients.

Key words: Dental care, health profile, sickle cell anemia

INTRODUCTION

Sickle cell anemia (SCA) is an inherited disease in individuals homozygous for hemoglobin S.[1,2] Previous studies have shown clinical alterations in the oral mucosa and dental mineralized tissues and delayed tooth eruption,[3] a high prevalence of dental caries,[4] poor occlusion,[5] and pulpal necrosis in healthy teeth[6] in patients with SCA. However, some studies have reported conflicting results as to the prevalence of caries and periodontal diseases.[7,8] Thus, this study was conducted to assess differences in the dental care provided to individuals with SCA depending on age using quantitative analysis of the procedures performed in the Center of Hematology and Hemotherapy in Maranhão (HEMOMAR).

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MATERIALS AND METHODS

This retrospective study used secondary data obtained from 574 dental records of patients with SCA treated or under treatment in the Dental Department of HEMOMAR from 2000 to 2011. The local Ethics Research Committee approved the study under protocol #0067182011-73.

A single calibrated examiner performed data collection ($\kappa = 0.91$). Data on gender (male and female), age, duration of dental treatment (<1 year; >1–4 years; >4–7 years; >7–11 years), types of dental procedures, and reasons for dental procedures were collected. The patients were stratified in respect to age as children (0–9 years), adolescents (10–19 years), young adults (20–40 years), adults (41–65 years), and the elderly (over 65 years).[9] Types of dental treatment included periodontal treatment (PT - yes or no), number of filled teeth (FT), number of extracted teeth (EX), and number of endodontically treated teeth (ET). The reasons for dental procedures included restorative treatment (caries), tooth extraction (periodontal disease, irreversible pulpitis, chronic apical periodontitis, crown destruction [more than 2/3 of the crown destroyed], failure of endodontic treatment, residual root, impacted teeth and not reported) and endodontic treatment (irreversible pulpitis, pulp necrosis, and chronic apical periodontitis).

All statistical analyses were performed using the SPSS software version 11 (IBM, NY, USA). The normality of the sample was tested using the Shapiro–Wilk test. The Kruskal–Wallis and Dunn's *post hoc* tests were performed to compare the FT, EX, and ET between age groups. The Chi-square test complemented by analysis of adjusted residuals was used to assess whether there were differences in the prevalence of PT between age groups. The Spearman's correlation was used to determine the correlation between age group and FT, EX, and ET. The level of significance was set for an alpha error of 5%.

RESULTS

Table 1 shows that the study population comprised predominantly of young adults (41.5%), males (53.8%), and patients under treatment for <1 year (51.2%).

Overall, 673 (100%) dental procedures were performed: 394 FT (58.6%), 241 EX (35.8%), and 38 ET (5.6%). Of the dental records analyzed, 66 (11.5%) of 574 patients were submitted to PT [Table 2].

The most common reasons for restorations and endodontic treatment were dental caries (100%) and irreversible pulpitis (55.6%), respectively. The main reasons for teeth extractions were residual roots (21.3%), chronic apical periodontitis (19.7%), and crown destruction (19.3%) [Table 3].

The variables FT, EX, and ET had an asymmetric distribution between age groups. Differences in the frequencies of FT, EX, ET, and PT according to age group are shown in Table 2. The median number of FT was significantly lower in children than in adults, young adults and adolescents ($P < 0.001$), and significantly higher in adults than in adolescents ($P = 0.028$). Children had a lower median number of EX than the young adults ($P = 0.013$) and adults ($P = 0.022$). The median numbers of ET in children and adolescents were lower than in young adults and adults ($P < 0.001$). There was a higher prevalence of PT in the older age groups ($P < 0.001$).

Strong positive correlations were found between age group and EX ($r = 0.93$; $P = 0.025$) and ET ($r = 0.92$; $P = 0.028$), and a weak positive correlation between age group and FT ($r = 0.153$; $P < 0.001$).

DISCUSSION

Significant differences in the type of dental treatments were identified in the diverse age groups of patients with SCA [Table 3]. There was a growing trend of

Table 1: Study population stratified by age group, sex, and time of dental treatment

Variables	*f*	%
Age group		
Children	137	23.9
Adolescents	178	31.0
Young adults	238	41.5
Adults	21	3.6
Sex		
Male	309	53.8
Female	265	46.2
Time of dental treatment (years)		
<1	294	51.2
>1-4	124	21.6
>4-7	69	12.0
>7-11	87	15.2
Total	574	100.0

São Luis, Maranhão, Brazil (2000-2011). *f*: Absolute frequency, %: Relative frequency

Table 2: Differences between types of dental procedures and age group

	Age group								P[a]
	Children		Adolescents		Young adults		Adults		
	μ (SD)	m (dIQ)	μ (SD)	m (dIQ)	μ (SD)	m (dIQ)	μ (SD)	m (dIQ)	
Number of filled teeth	2.04 (3.14)	0 (3)	2.72 (2.39)	2 (3)	3.28 (3.32)	3 (5)	5.1 (4.7)	4 (4)	<0.05[bcde]
Number of extracted teeth	0.63 (1.08)	0 (1)	0.82 (1.41)	0 (1)	1.09 (1.73)	0 (2)	1.24 (1.73)	1 (2)	<0.05[cd]
Number of endodontically treated teeth	0.02 (0.15)	0 (0)	0.06 (0.32)	0 (0)	0.13 (0.43)	0 (1)	0.38 (0.92)	0 (1)	<0.001[cdeg]
	f	%	*f*	%	*f*	%	*f*	%	P[h]
Periodontal treatment									
Yes	5	3.6	14	4.5	39*	16.4*	8*	38.1*	<0.001
No	132	96.4	164	95.5	199	83.6	13	61.9	
Total	137	100	178	100	238	100	21	100	

São Luis, Maranhão, Brazil (2000-2011). [a]Kruskal-Wallis and Dunn's *post hoc* test, [b]Adolescent≠children, [c]Young adults≠children, [d]Adults≠children, [e]Adults≠adolescents, [f]Adults≠young adults, [g]Young adults≠adolescents, [h]Chi-square test, *Local association. *f*: Absolute frequency, %: Relative frequency, μ: Mean, SD: Standard deviation, m: Median, dIQ: Interquartile deviation

Table 3: Reasons for dental procedures by age group

Reason	Age group								Total	
	Children		Adolescents		Young adults		Adults			
	f	%	*f*	%	*f*	%	*f*	%	*f*	%
Number of filled teeth										
Caries	69	50.4	133	74.7	175	73.5	17	81	394	100
Number of extracted teeth										
Periodontal disease	0	0	0	0	2	1.9	0	0	2	0.8
Irreversible pulpitis	0	0	1	1.5	1	0.9	0	0	2	0.8
CAP	14	29.2	13	18.8	16	14.8	4	28.6	47	19.7
Crown destruction	8	16.6	15	21.7	21	19.4	2	14.3	46	19.3
Failure of ET	0	0	0	0	2	1.9	0	0	2	0.8
Residual root	7	11.6	8	11.6	30	27.8	6	42.8	51	21.3
Impacted teeth	2	2.9	2	2.9	3	2.8	0	0	7	2.9
Not reported	17	43.5	30	43.5	30	30.5	2	14.3	82	34.4
ET										
Irreversible pulpitis	3	100	4	50	11	52.4	2	50	20	55.6
pulp necrosis	0	0	1	12.5	7	33.3	0	0	8	22.2
CAP	0	0	3	37.5	3	14.3	2	50	8	22.2

São Luis, Maranhão, Brazil (2000-2011). *f*: Absolute frequency, %: Relative frequency, CAP: Chronic apical periodontitis, ET: Endodontic treatment

curative dental procedures performed as patients aged such as FT, EX, ET, and PT. However till date, there are no studies correlating the type or frequency of dental procedures to age in this population. This cumulative trend of oral disease is also observed in the general Brazilian population, especially in respect to tooth decay. Based on the results of the National Oral Health Survey in 2010 (SB Brasil), in 35–44-year-old individuals, the mean decayed, missing, and FT index is 16.75, indicating a possible continuation of the severity of caries in the adult population.[10] The absence of an oral health policy focused on this population may be responsible for the continuation of the high rates of dental caries. The lack of access to preventive measures and assistance or the inefficient and poor quality treatment possibly increase the complexity of the problem.[11]

In Brazil, babies with SCA have been identified through the National Neonatal Screening Program, better known as the "heel prick test," since 2001. We believe that dental care is neglected due to systemic complications of the disease. Probably this is the main cause of the increasing number of dental curative procedures with age seen in this study. Moreover, this is related to the difficulty of SCA patients in accessing primary healthcare services through the Brazilian National Health Service (NHS) because they have a genetic condition and are generally black.[12-14] Genetic diseases do not have set guidelines in primary care. The focus of these patients is on secondary care, often in hematology centers.[10] The black population in Brazil still live in a socially vulnerable situation with difficulties in accessing health services.[13,14]

Since the effectiveness of dental care is one prerequisite in the planning of local oral healthcare services,[15] we believe that the quantitative analysis of the types of dental procedures performed over an 11-year period in the HEMOMAR Dental Service might be an indirect measure to evaluate the oral health profile of this population.

There were relatively few adults in this population-based sample [Table 1]. According to Platt *et al.* (1994)[16] and Alves (1996),[17] health treatments are more commonly sought after by young individuals with SCA because these patients do not usually reach old age. These authors reported that patients with SCA die prematurely due to complications of the disease. According to Platt *et al*. (1994),[16] a decrease of 25–30 years in life expectancy is observed in the survival curve of patients with SCA compared to the general population.

Restorative treatment was the most common type of conduct in this population. Previous studies found contrary results, that is, individuals with SCA presented with decay but they were not treated.[8,18,19] According to these authors, this is a consequence of poor adherence to regular dental treatment due to lack of opportunity resulting from their numerous hospitalizations for disease-related complications throughout their lives.

Bacterial infections are considered the main cause of child mortality in this population.[20] A plausible explanation for this is the fact that children with SCA are easily infected by bacteria.[21] As a measure to prevent infant death, people with SCA are submitted to prolonged treatment with antibiotics such as penicillin since early childhood, which significantly reduces the number of *Streptococcus mutans* and *Aggregatibacter actinomycetemcomitans* in the oral cavity; this seems to reduce the number of caries and periodontal diseases.[22] This may have contributed to the reduced number of curative dental procedures in children in this study.

Another relevant aspect is that only 11.5% of the sample was submitted to PT and of this total, a significant percentage was distributed across the age groups of young adults and adults ($P < 0.001$). This finding corroborates the results of Passos *et al*.,[8] who after multivariate logistic regression analysis, showed that older individuals with SCA are at higher risk of developing periodontal pockets. These authors reported that age and oral hygiene habits are considered greater risk factors for the development of periodontal diseases compared to the direct systemic impact of the disease.

A low number of FT, EX, and ET were observed in children and adolescents and a high number of EX and ET were seen in older age groups. These results demonstrate that the type of oral health care adopted at HEMOMAR is a curative hegemonic model. In Brazil, this dental care model was adopted for school-aged individuals in the late 1950s. In the present study, it was found that even making use of dental care services, young adults and adults with SCA had a high number of FT ($r = 0.153$; $P < 0.001$), EX ($r = 0.93$; $P = 0.025$), ET ($r = 0.92$; $P = 0.028$) and were also frequently submitted to PT ($P < 0.001$).[23]

It is speculated that, in most of the cases, these procedures could have been avoided if the care model adopted by the institution had been based on prevention and promotion of health, which encourages training and oral health practices focused on conducts that have higher effectiveness and lower costs for the NHS than a curative model.[24]

There are some public policies aimed at the population with SCA in Brazil. However, data from our study show that half of the population was under treatment for <1 year and most procedures were entirely curative. This confirms the vulnerability of these individuals to dental problems and consequently the need for assistance to treat problems. On the other hand, it is possible that dental care services do not prioritize prevention and promotion measures. In addition, individuals with SCA have psychological and social obstacles, which, added to the chronic nature of the disease, interfere in their search and access to treatment,[23] possibly leading this population to the underuse of the oral health services offered by primary healthcare in the NHS.

The high frequency of teeth extractions (35.8%) in this study is explained by the clinical and radiographic diagnosis of chronic apical periodontitis as a sequela of pulp necrosis, possibly due to repeated vaso-occlusive crises that impair the microcirculation of the dental pulp [Table 3].[6,25] It is believed that the fear that endodontic infections may evolve to osteomyelitis, and the impracticability of crown reconstruction through direct restorations, which are not offered by the Brazilian NHS, are the main reasons for tooth extraction.[6,25]

Although the health service of this study has a good records system, information bias may be a limitation in all research that uses secondary data. In the current study, it was not possible to establish any relationship between the underlying disease and dental conditions because there were no data in respect to sickle cell disease in the medical records on the past and current medical histories of patients, only the type of hemoglobinopathy.

Thus, due to the lack of publications on this topic, this study has much to contribute to the literature. In addition, as a population-based study, it allows a good generalization; or rather its data is representative of the population of patients with SCA treated in a public healthcare service. Furthermore, the probability of selection bias was reduced, as this research did not include SCA patients treated in the private sector.

CONCLUSIONS

Dental caries, endodontic, and PTs are more frequent with advancing age. The procedures offered are still mainly guided by curative strategies which indicate the need for systematic preventive treatment protocols for dental caries and their sequels and periodontal diseases in this population.

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Conflicts of interest
There are no conflicts of interest.

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Healthy Dental Pulp Oxygen Saturation Rates in Subjects with Homozygous Sickle Cell Anemia: A Cross-Sectional Study Nested in a Cohort

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Abstract

Introduction: To compare the percentage of arterial oxygen saturation (SpO_2) in healthy teeth with confirmed pulp vitality between individuals with sickle cell anemia (HbSS) and normal hemoglobin A (HbAA). **Methods:** This is a cross-sectional study nested within a cohort. Samples (n = 2543) comprised teeth with intact crowns and pulp vitality confirmed by thermal sensitivity tests and no history of caries, periodontal disease, or dental trauma. A total of 728 teeth of 113 individuals with HbSS and 1815 teeth of 246 individuals with HbAA were evaluated. Data analysis was performed using the χ^2 and Mann-Whitney tests and Spearman correlation analysis (α = 0.05). **Results:** The study groups were comparable in terms of age, race, and sex (P > .05). Subjects with HbSS exhibited lower median SpO_2 levels in the body and upper teeth, excluding canines, than subjects with HbAA (P < .05). There were no significant differences in the evaluated parameters between the 2 groups (P > .05). **Conclusion:** Compared with individuals with HbAA, those with HbSS exhibited lower SpO_2 in maxillary teeth with confirmed pulp vitality, except in the canines. There was no correlation between SpO_2 levels of the body and dental pulp in individuals with HbSS or HbAA. *(J Endod 2017;■:1–4)*

Key Words

Dental pulp, pulse oximetry, sickle cell anemia

Significance

The clinical relevance of this study is the need to establish the arterial oxygen saturation level for healthy dental pulp in teeth with intact crowns in individuals with sickle cell anemia by means of pulse oximetry adapted for dentistry.

Sickle cell anemia (SCA) is a structural hemoglobinopathy that occurs because of a point mutation in the β-globin gene of normal hemoglobin (HbA), resulting in an altered Hb molecule (HbS) (1). In cases of prolonged hypoxemia, HbS polymerizes and, consequently, causes the sickling of red blood cells (RBCs) and increases their adhesion to the vascular endothelium. This vicious cycle of hypoxemia and sickling of RBCs induces systemic complications, such as local vasoconstriction and episodes of pain, which can result in injury to vital organs (2). In the oral cavity, this condition might cause ischemia and necrosis of the facial bones, triggering osteomyelitis without dental cause, especially in the mandible (3, 4). It has been demonstrated that vaso-occlusion of the microcirculation of dental pulp in healthy teeth results in pulpitis (5, 6) and even pulp necrosis (7–10).

Percentage of arterial oxygen saturation (SpO_2) in the body, currently considered the fifth vital sign, is measured with pulse oximetry (PO) (11), which is based on 2 basic principles: spectrophotometry and plethysmography. Spectrophotometry involves measurement of the amount of light reflected solely by oxyhemoglobin in capillaries, and plethysmography involves measurement of the volume of arterial blood in tissues (12). PO has been adapted in dentistry for the estimation of pulp vitality in various populations, such as pediatric patients (13), young individuals with tooth trauma (14, 15), healthy adults (16), patients scheduled for radiotherapy for treatment of intraocular or oropharyngeal tumors (17), and patients with SCA (10).

PO involves the calculation of the SpO_2 level of the body from measurements of partial arterial oxygen pressure using an HbA dissociation curve obtained in subjects with normal Hb phenotype (HbAA). However, as individuals with SCA (HbSS) achieve <90% SpO_2 (18), results obtained in this manner in such individuals can be spurious (11) because of the possibility that the curve might deviate to the right.

Thus, as PO adapted for dentistry is considered a reliable tool for the estimation of pulp vitality (12, 14, 15, 19), the null hypotheses tested in this study were as follows:

1. There is no difference in SpO_2 levels of the body or healthy teeth with confirmed pulp vitality between individuals with HbSS and HbAA.
2. There is no correlation between SpO_2 levels of the body and healthy teeth among individuals with HbSS or HbAA.

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Materials and Methods

This cross-sectional study nested within a cohort (10) was approved by the local ethics research committee (protocol number: 23115 004993/2010-71). The sample comprised 2543 healthy teeth with confirmed pulp vitality and no history of caries, periodontal diseases, or dental trauma from 113 and 226 individuals with (HbSS) and without (HbAA) SCA, respectively. All participants signed informed consent forms.

The cohort study was conducted in 2 phases, 1 retrospective and 1 prospective. The retrospective phase involved selection of study participants with SCA diagnosed on the basis of hemoglobin electrophoresis findings at birth, retrieved from medical records. The prospective phase involved evaluation of the main outcomes (SpO_2 in the body and healthy teeth) determined with PO. Teeth were categorized into subgroups (maxillary and mandibular central and lateral incisors, maxillary and mandibular canines, premolars, and upper and lower molars). Covariates were participant age in years, sex, and self-reported race (mulatto, black, and white).

All subjects with SCA (n = 1600) enrolled at the Social Work Sector of the Hematology and Hemotherapy Center of Maranhão (HEMOMAR), São Luís, MA, Brazil, were eligible for inclusion in the SCA group. Participants were selected by a simple random draw from among subjects without a history of vaso-occlusive crises in the previous 6 months. Relatives of individuals with SCA, who did not have any systemic conditions, including SCA, were selected to form the control group. All participants were older than 16 years and possessed at least 1 healthy tooth with an intact crown. Individuals with inferior alveolar nerve paresthesia in the 6 months leading up to this study, teeth affected by dental trauma, or pulp necrosis in healthy teeth were excluded from both groups.

We estimated that a sample of 2543 teeth, including 728 and 1815 teeth of individuals with HbSS and HbAA, respectively, would have a 96.6% power to identify the differences in mean SpO_2 levels of teeth between the 2 study groups, considering an α error of 5% and 1:2 ratio of population between the 2 groups. The mean values of SpO_2 for pulp vitality of teeth in subjects with HbSS and HbAA were set at 85.9% $\pm$ 5.25% and 88.4% $\pm$ 5.11%, respectively. This information is based in a previous study (10).

The reference measurements of SpO_2 were initially recorded at the right index finger. The body and pulp SpO_2 levels were measured using a PO (MD300A; IMFtec Tecnologia para Saúde LTDA, São Paulo, SP, Brazil). The oximeter sensor has 2 diodes, a receiver and a transmitter. The sensor accuracy reported by the manufacturer is 70% to 100%. The pulp SpO2 levels were measured using an oximeter sensor adapted for the human dental anatomy (10). During measurement of pulp SpO_2 level, the reflector light was turned off, and the diodes were held parallel to each other with the thumb and forefinger of the right hand, so that the receiver received the light transmitted through the tooth by the transmitter. Patients were instructed to keep their head still so as to not influence the oximeter readings. This test was repeated 3 times, and the median value of the 3 readings was used for further analysis. Pulp vitality was assessed by Cold Sensitivity Test (CST). The CST was performed 5 minutes after PO. Additionally, thermal sensitivity to cold was evaluated with dichlorodifluoromethane gas (Endo Frost; Vigodent SA Industria e Comercio, Bonsucesso, Rio de Janeiro, Brazil) by means of a microbrush applied to the cervical region of the vestibular face of the dental crown for4s.

Statistical Analysis

The Shapiro–Wilk test was used to evaluate the normality of variables. The χ^2 test was used to determine the similarities in participant age, sex, and race between the study groups. The Mann-Whitney test was used for comparison of SpO_2 levels among the dental subgroups in the HbSS and HbAA groups. Possible associations between SpO_2 levels of the body and dental pulp between the study groups were assessed with Spearman correlation analysis. The level of significance was set at 5%. All analyses were performed using the Stata computer program version 9.0 (Stata Corp, College Station, TX).

Results

The groups were statistically comparable in terms of participant age, race, and sex (P > .05). The mean participant ages in the HbSS and HbAA groups were 28.74 $\pm$ 11.38 and 27.47 $\pm$ 10.22 years, respectively. Among participants in the HbSS and HbAA groups, 62.0% and 61.5% were black individuals and 50.4% and 57.1% were male individuals, respectively.

A total of 2543 teeth were evaluated. The SpO_2 values of the body and dental pulp were asymmetrical in both groups. The SpO_2 levels of the body, maxillary central and lateral incisors, maxillary premolars, and molars in subjects with HbSS were lower than those in subjects with HbAA (P < .05; Table 1). There was no significant difference for SpO_2 values of maxillary canines and mandibular teeth between study groups (P > .05; Table 1).

There was no correlation between SpO_2 levels of the body and dental pulp in study groups (P > .05; Table 2).

Discussion

In this study, the first null hypothesis was partially rejected on the basis of the findings, indicating differences in SpO_2 levels of the body and dental pulp of the maxillary teeth, except for canines, between the HbSS and HbAA groups (P < .05; Table 1). The difference in SpO_2 levels of the body between the 2 groups might be explained by methemoglobinemia (presence of methemoglobin in blood at higher levels than normal) in individuals with HbSS. Methemoglobin is a form of HbA that does not bind to oxygen (20, 21); methemoglobinemia can lead to tissue hypoxia characterized by SpO_2 levels of ≤94% (22–24).

The relationship between tissue hypoxia and infection in individuals with HbSS is bidirectional, because the state of hypoxia leads to the proliferation of microorganisms, just as an infection can trigger a vaso-occlusion and consequently tissue hypoxia (25). In addition to tissue hypoxia, the major reason for vulnerability to serious infections in these individuals is splenic dysfunction (26, 27), which results in decreased production of interleukin-4 and immunoglobulin M and impairment of B-lymphocyte function (26). On the other hand, infection induces increased fibrinogen production and stimulates the adhesion of sickled RBCs to the vascular endothelium, thereby promoting vasoconstriction and tissue hypoxia (27, 28). This results in tissue damage, which can manifest as painful crises and damage to vital organs (27), including infection of the periodontal membrane, vaso-occlusion, and microinfarction of the vascular–nervous supply to the dental pulp (4).

The present findings revealed no differences in SpO_2 levels of the upper canines or lower-jaw subgroups between the HbAA and HbSS groups (Table 1). The relatively low SpO_2 in the maxillary teeth of individuals with HbSS might be attributable to the formation of microthrombos as a result of vaso-occlusion due to the accumulation of sickled RBCs in the large medullary spaces of bones. This phenomenon is probably less frequent in the region of the upper canines because, of all maxillary teeth, the superior canines in relation to the other teeth are surrounded by denser and more compact medullary bone, adapted to withstand the impact of the horizontal forces that occur during the eccentric movements of the mandible (29). The bone marrow of individuals with HbSS suffers from hypertrophy due to the increased production of new RBCs to compensate for their short life (28, 30); considering

TABLE 1. Comparison of SpO_2 Levels of the Body and Dental Pulp between the Study Groups

SpO_2	HbAA (*n* = 1815)				HbSS (*n* = 728)				*P**
	n	M (%)	Min (%)	Max (%)	*n*	M (%)	Min (%)	Max (%)	
Index finger	226	87	70	92	113	85	70	96	**.022**
Teeth									
Maxillary									
Central incisors	102	89	84	93	42	78	73	91	**.005**
Lateral incisors	132	87	82	91	36	79	73	93	**.005**
Canines	174	86	74	94	87	87	74	92	.073
Premolars	234	91	67	95	94	87	85	95	**< .001**
Molars	191	89	70	98	81	75	70	94	**< .001**
Mandibular									
Central incisors	74	83	79	91	36	86	79	91	.074
Lateral incisors	113	91	85	99	16	93	89	99	.070
Canines	200	90	77	99	120	93	77	97	.309
Premolars	497	88	82	96	190	88	83	96	.539
Molars	96	89	85	93	24	89	85	93	.872

HbAA, normal hemoglobin A; HbSS, sickle cell anemia; M, median; Max, maximum; Min, minimum; *n*, absolute frequency; SpO_2, percentage of arterial oxygen saturation.

*Mann-Whitney test. Values in bold indicate statistical significance.

that the maxilla is a predominantly medullary bone, this phenomenon could explain the formation of large spaces in the bone marrow of the maxilla. This altered bone pattern is considered to be one of the main manifestations of odontological interest in SCA (30–32).

In contrast to the first, our second null hypothesis was proven true. There was no correlation between the SpO_2 levels of the body and dental subgroups in the HbAA or HbSS group. These findings support the results of Pozzobon et al. (13), Gopikrishna et al. (14), and Goho (33). This finding might be attributable to the interference of the mineralized tissues surrounding the dental pulp, including the enamel prisms and dentin, with the refraction of the infrared light emitted by the PO diode (13, 33).

In incisors and canines, SpO_2 levels of >87% indicate pulp vitality (34). However, it should be remembered that this cutoff point was determined on the basis of dental pulp SpO_2 levels in individuals with HbAA. To date, the oxygen saturation pattern of the dental pulp in individuals with HbSS remains unknown. The present findings revealed a median SpO_2 of $\leq$ 79% in the maxillary central and lateral incisors and $\leq$75% in the maxillary molars of individuals with HbSS, which is approximately 10% lower than that in individuals with HbAA. Considering that individuals with HbSS suffer from chronic tissue hypoxemia throughout their lives, this value is consistent with the pathophysiology of SCA. In addition, participants with HbSS in this study received hematological treatment, including continuous administration of vitamins A and B12, medication with ferritin and hydroxyurea, and blood transfusion, for minimizing the incidence of vaso-occlusive events.

TABLE 2. Correlation between SpO_2 Levels of the Body (Index Finger) and Dental Pulp in the Study Groups

SpO_2 of the body versus dental pulp	HbAA		HbSS	
	r	*P**	*r*	*P**
Maxillary				
Central incisors	0.104	.111	0.072	.534
Lateral incisors	0.105	.125	0.073	.535
Canines	−0.290	.701	0.510	.642
Premolars	0.114	.081	−0.220	.063
Molars	0.240	.061	−0.021	.836
Mandibular				
Central incisors	0.025	.731	0.043	.758
Lateral incisors	0.022	.728	0.050	.761
Canines	0.260	.713	0.110	.909
Premolars	0.021	.646	0.052	.475
Molars	0.179	.109	−0.074	.731

r, correlation coefficient; SpO_2, percentage of arterial oxygen saturation.

*Spearman's correlation analysis.

The strength of this study is its originality in establishing the SpO_2 level of dental pulp in healthy teeth in individuals with HbSS as well as its large sample size and the inclusion of premolars and molars among the investigated tooth samples. Bruno et al. (34) performed a systematic review with meta-analysis aimed at estimating the median SpO_2 levels of healthy dental pulp in central and lateral incisors and canines; only 6 studies, including a total of 472 teeth (central incisors, 288; lateral incisors, 90; and canines, 94), were included in the statistical analysis, which corresponded to just 19% of the present sample size.

Although individuals with HbAA and HbSS in this study were not paired, the 2 study groups were comparable in terms of race, sex, and participant age; therefore, the possibility of selection bias was low. As the HbAA group was populated with the relatives of individuals with HbSS, the 2 study groups were derived from the same population, which minimized the potential for bias that might have arisen had the groups been selected from different sources.

PO in dentistry is a reliable, nonsubjective method that has been validated for clinical diagnosis of pulp vitality (33–35). However, dental professionals should always be attentive to individuals who do not have "normal" oxygen saturation levels (95%–100%) (23), such as those with HbSS (SpO_2 $\leq$90%) (23, 24), who, as demonstrated in the present study, exhibit lower-than-normal SpO_2 levels in the body and maxillary teeth.

Conclusion

In conclusion, individuals with HbSS exhibit relatively low SpO_2 levels in the body and maxillary teeth with confirmed pulp vitality, except in the canines. There is no correlation between the SpO_2 levels of the body and dental pulp in individuals with HbSS or HbAA.

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ORIGINAL ARTICLE

WILEY ORAL DISEASES

Association of sickle cell haemoglobinopathies with dental and jaw bone abnormalities

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Objective: To estimate the association between sickle cell anaemia and trait with dental and jaw bone abnormalities.

Subjects and Methods: Subjects (n = 369) were allocated to three groups: sickle cell anaemia, trait and control. Dental shape, number, size and position and changes in pulp chamber, root and periapex were analysed by intra-oral periapical radiographs. Integrity of lamina dura, quality of cancellous bone and bone trabeculation were also evaluated. Prevalence ratios (PR) were calculated (α = 0.05).

Results: Sickle cell anaemia had higher prevalence (PR:8.31) and number of teeth (PR:13.40) with external resorption; higher number of teeth with pulp calcification; partial and total loss of lamina dura; and higher prevalence of changes in trabecular structure of maxilla (PR:6.45) and mandible (PR:5.34). Sickle cell trait showed higher prevalence (PR:1.26) and higher number of teeth (PR:1.98) with partial loss of lamina dura; higher number of teeth with hypercementosis, changes in shape, size, periapex, total loss of lamina dura; and higher prevalence of changes in mandibular trabecular bone (PR:1.43).

Conclusion: Pulp calcification and external resorption of the root were the most frequent dental alterations in sickle cell anaemia group, while in trait was higher frequency of changes in shape, size, periapex and root. Jaw bone changes were most prevalent in both homozygous and heterozygous subjects.

KEYWORDS
dental radiography, maxillofacial abnormalities, sickle cell anaemia, sickle cell trait

1 | INTRODUCTION

Sickle cell anaemia (SCA) is a genetic condition characterized by vaso-occlusive episodes and infectious events (Kato, Hebbel, Steinberg, & Gladwin, 2009). This haemoglobinopathy is the result of a molecular modification causing the substitution of glutamic acid for valine in the beta chain of haemoglobin, thus leading to the production of modified haemoglobin (Hb S) (Kato et al., 2009; Steinberg, 2009). In homozygotes, the presence of Hb S instead of the normal haemoglobin (Hb A) is genetically inherited from both parents and characterizes SCA (Steinberg, 2008). Another condition considered asymptomatic and associated with the presence of Hb S is sickle cell trait (SCT); this condition is caused by heterozygous inheritance in which an individual receives the recessive gene for Hb S from one parent and a dominant gene for Hb A from the other (Goldsmith et al., 2012; Steinberg, 2008; Tsaras et al., 2009).

In hypoxic conditions, biochemical changes in the Hb molecule trigger the formation of polymers that lead to a change in the biconcave discoid configuration of red blood cells, thus causing a shape similar to a sickle (Ferreira et al., 2016; Kato et al., 2009). Consequently, sickled cells become less flexible and more adherent to the vascular endothelium, blocking capillaries and restricting blood flow to multiple organs and systems (Acharya, 2015; Kato et al., 2009). These vaso-occlusive events result in ischaemia, infarction and tissue damage, which can lead to significant systemic complications, such as pain crises, stroke, avascular necrosis of the bone, osteopenia, osteoporosis and high susceptibility to infections (Kato et al., 2009; Kelleher, Bishop, & Briggs, 1996).

The most common findings in the oral cavity indicated in the literature are delayed tooth eruption (Acharya, 2015), osteomyelitis (Shroyer, Lew, Abreo, & Unhold, 1991), hypomaturation and hypomineralization of both the enamel and dentin (Soni, 1966), malocclusion (Costa, Carvalho, Souza Sde, & Thomaz, 2015) and asymptomatic pulp necrosis (Costa, Thomaz, & Souza, 2013). Dental changes, such as pulp calcification and hypercementosis, have been reported and observed on radiographic images (Bishop, Briggs, & Kelleher, 1995; Soni, 1966; Taylor, Nowak, Giller, & Casamassimo, 1995). Another change in SCA that can be verified radiographically is the decrease in maxillary and mandibular bone radiodensities and the formation of a coarse trabecular pattern described as "ladder shaped" (Neves et al., 2011). This change is attributed to erythroblastic hyperplasia resulting from the vaso-occlusive phenomenon and compensatory bone expansion (Costa, de Carvalho, Thomaz, & Sousa Sde, 2012).

Although individuals with SCT are considered asymptomatic, when exposed to low oxygen conditions, they may suffer vaso-occlusion and potentially present clinical complications similar to SCA (de Carvalho, Thomaz, Alves, & Souza, 2015; Key & Derebail, 2010), such as trabecular bone changes (Gillis & West, 2004). However, population-based studies that evaluated the associations of SCA and SCT with dental and bone changes were not found. Some of the studies found were case reports (Gillis & West, 2004; Kelleher et al., 1996), literature reviews (Javed et al., 2013; Kelleher et al., 1996) and radiographic evaluations with small sample sizes (Sanger & Bystrom, 1977). One study found a correlation between bone changes and clinical severity of SCA (Neves et al., 2012), but it did not include a control group. Therefore, these study designs are not appropriate to estimate associations between exposure and outcome.

Individuals with SCA or SCT are considered to have significantly higher frequencies of dental and bone changes than those without these conditions. This hypothesis was investigated by radiographic features that showed changes that could not be detected clinically. This study aimed to estimate the association of SCA and SCT with dental and jaw bone abnormalities.

2 | SUBJECTS AND METHODS

2.1 | Study design

This cohort study was conducted in two phases: a retrospective phase and a prospective phase. The exposures (SCA and SCT) and the outcomes of interest (dental and jaw bone abnormalities) occurred before the study. Subjects were selected on the basis of the retrospective investigation of the diagnosis of exposure, which was obtained from hospital records. Therefore, the exposures were identified retrospectively, and the outcomes were diagnosed prospectively. The study participants were grouped on the basis of exposure status and were divided into two exposed groups and one non-exposed group. Patients with SCA and SCT were selected to form the two exposed groups, and patients without SCA or SCT were selected to form the non-exposed group. For all groups, both SCA and SCT were diagnosed by haemoglobin electrophoresis at birth. The selected individuals were contacted and invited to attend the dental department of HEMOMAR (Supervisão de Hematologia e Hemoterapia do Maranhão) in São Luís, Brazil, from August 2012 to October 2013. At the time, they were interviewed, examined and X-rayed. This study was approved by the local Research Ethics Committee (Protocol No. 472.943/2013) and conducted according to the norms of the Declaration of Helsinki. The participants voluntarily signed informed consent forms.

2.2 | Sample calculation

A minimum sample size of 103 subjects per group (n = 309) was estimated at a ratio of 1:1:1 among the SCA, SCT and control groups to obtain a power of 85% to detect prevalence ratios (PR) of 2.5, given a prevalence of 11% of bone changes and a two-sided confidence interval of 95%. To compensate for possible loss of information, 20% more individuals were added to the calculated sample size to obtain 123 people in total in each group (n = 369).

2.3 | Selection of study participants

The SCA subjects were selected from a population of 1,600 potentially eligible individuals who received care in HEMOMAR through a simple random sampling without replacement. The SCT group was composed of the relatives of the SCA subjects who were recruited. The control group consisted of the neighbours of the exposed subjects who did not have SCA or SCT to constitute a total sample exposed to the same environmental factors. The selection of SCT and control subjects was related to the inclusion of the SCA subjects. The 426 subjects with or without SCA and SCT were recruited from the medical records in accordance with the sample size.

Subjects with other systemic conditions, such as diabetes (n = 6), obesity (n = 1) and chronic kidney disease (n = 3) that could represent additional risk factors for bone changes and/or prevent suitable radiographic evaluation, were not included in the study. Furthermore, individuals who underwent periodontal treatment, those who had taken antibiotics/anti-inflammatory drugs within the previous 6 months (n = 9), edentulous subjects (n = 10) and expectant or breastfeeding women were excluded (n = 7). Individuals with more severe diseases and potentially higher risk of developing oral problems were also excluded from this study (n = 10). From among the 381 eligible individuals, five individuals with SCA and seven from the control group declined to participle. Thus, 123 subjects with SCA, 123 with SCT and 123 without SCA or SCT (control) were obtained.

2.4 | Data collection

Demographic data were obtained by two blinded, previously calibrated examiners (k = 0.87) using a structured questionnaire to attain the name, gender, self-reported skin colour, age in years, socioeconomic class (Brazilian Market Research Association, 2015), personal habits, general health status and issues related to oral health.

To obtain images, intra-oral periapical radiographs were taken of all sextants using a parallel technique with conventional radiographic positioners. Insight no 2 X-ray films (Kodak Brazilian Trade Products Image and Services Ltda., Manaus, Brazil) and an XRM X-ray apparatus (Rhos, Rio de Janeiro, RJ, Brazil) operating at 60 kVp and 10 mA were used. Radiographic processing was performed in a portable box using the time–temperature method. The obtained radiographic images were digitized using a scanner (XR-700 X-ray film USB Reader TPC Advanced Technology, Inc., CA, USA) and stored on a computer with the Windows® operating system (Microsoft, Redmond, WA, USA) in JPEG format. Dental and jaw bone changes were analysed using the ImageJ® software version 1:47 (National Institutes of Health—NIH, Bethesda, Maryland, USA). Radiographic evaluations were performed in triplicate by three matched blind examiners with clinical and academic experience in radiographic studies, who were trained during 2 months. In case of disagreement, a new evaluation was carried out by these examiners until consensus was reached. To verify the inter-examiner agreement, 140 intra-oral periapical radiographs (full mouth series of ten patients) were evaluated blindly in triplicate at different times by the three independent examiners. These 140 radiographs were included in the sample, avoiding unnecessary exposures due to ethical reasons. The outcome variables considered during the calibration sessions and mean kappa values for inter- and intra-examiner agreement for detection of dental and bone changes are given in (Tables S1 and S2).

2.5 | Evaluation of dental abnormalities

Possible changes in the pulp chamber (atresia, ~~pulp canal calcification,~~ pulp nodules and taurodontism), root surface (hypercementosis and internal/external root resorption) and periapex [suggestive radiolucent image of apical periodontitis of endodontic origin, radiolucent image with disruption of the lamina dura (LD) and radiopaque image] were investigated (Neville, Damm, Allen, & Chi, 2015a,b). Changes in shape (fusion, gemination, *dens in dente*, concrescence and dilaceration), number (presence of supernumerary teeth and agenesis/anodontia), size (microdontia and macrodontia) and position (labial version/lingual version and impacted teeth) were also examined (Neville et al., 2015a). These outcomes were analysed in two ways: (i) prevalence of the dental change (categorized as having or not having the abnormalities) and (ii) number of teeth with each abnormality per affected individual. In all analyses, the individuals were the observation units.

2.6 | Evaluation of jaw bone abnormalities

The following radiographic parameters were considered to identify jaw bone changes: (i) integrity of the LD and (ii) bone marrow. The following radiographic patterns were considered in the assessment of the LD: loss of clarity and continuity, total or partial loss and increased thickness (Neville, Damm, Allen, & Chi, 2015c). After analysis, LD was classified as altered (total or partial loss and thickening) or unchanged (linear image on the proximal surfaces). The degree of bone trabeculation was analysed and classified as normal or altered [step-ladder, spider web or hair-on-end appearance] (Azam & Bhatti, 2006; Mourshed & Tuckson, 1980). Changes in LD were also analysed as binary (yes or not) and discrete (number of abnormalities per affected individual) outcomes, whereas changes in bone trabeculation were analysed only as binary variables.

2.7 | Statistical analysis

The Shapiro–Wilk test and graphical distribution histograms and box plots were adopted to assess the normal distribution of data. Absolute frequencies and percentages were estimated for categorical variables and means ± standard deviation ($x \pm SD$) and median ± interquartile deviations ($m \pm$ Q1–Q3) for numerical variables.

The comparison of frequencies among the groups was performed using the Kruskal–Wallis, Fisher exact and chi-square tests. To estimate the associations of interest, regression analyses were conducted using a stepwise modelling strategy. PR, unadjusted and adjusted 95% confidence intervals (95% CI) were calculated using Poisson regressions. Covariates with statistical significance of 20% were selected for multivariate models of unadjusted associations between outcomes (dental and bone changes). All analyses were performed using Stata version 11.0 (Stata Corp., College Station, Texas, USA). A level of significance of 5% was adopted.

3 | RESULTS

The data obtained showed an asymmetric distribution. Each group was composed of 123 individuals. The median (±Q1–Q3) ages of the three groups were as follows: SCA (17 ± 13 years), SCT (33 ± 8 years) and control (36 ± 19 years). The sample was composed mostly of women (79.7%) who lived in the countryside (52.3%), had a socioeconomic class of C (55.7%) and did not regularly visit a dentist (60.7%) (Table 1).

Changes in the pulp chamber were the most prevalent dental abnormalities in the three groups, followed by changes in the root surface and in the periapex. There was no difference in the prevalence of dental changes between groups ($p > .05$; Table 2). Among bone changes, partial loss of LD was more prevalent in SCT group ($p = .005$) and alterations in the trabecular bone (maxilla and mandible) were more prevalent in the SCA group ($p < .001$) (Table 2). Mean (±standard deviation) and median (±interquartile range) of the number of teeth with dental and bone changes by group are also shown in the Supporting information. Individuals with SCT had higher median values of partial loss of LD (1.00 ± 0.00–5.00; $p < .001$) than those in the other groups. The remaining bone and dental changes showed no statistically significant differences among the groups (Table S3).

In the unadjusted analyses, there was no difference in the prevalence of dental changes between groups. However, when considering the outcome as a counting of teeth with changes per person, SCA had a lower number of teeth with pulp canal obliteration and a higher number of teeth with external resorption than the control group. SCT was

TABLE 1 Sociodemographic characteristics, general health status and oral health by group

	Total sample		Exposed groups		Non-exposed group	
Variables	***N***	**%[a]**	**SCA (%)[b]**	**SCT (%)[b]**	**Control (%)[b]**	***p*-value**
Age						**<.001[c]**
<20 years	86	23.3	81.4	5.8	12.8	
20–29 year	84	22.8	36.9	28.6	34.5	
30–39 year	120	32.5	12.5	58.3	29.2	
≥40 anos	79	21.4	8.9	30.5	60.8	
Gender						**<.001[c]**
Male	75	20.3	58.7	14.7	26.7	
Female	294	79.7	26.9	38.1	35	
Self-reported skin colour						**.002[c]**
Caucasian	54	14.6	14.8	48.2	37	
Black	104	28.2	46.1	26.0	27.9	
Others	211	57.2	31.8	33.2	35	
Place of residence						**<.001[c]**
Capital	176	47.7	29	18.2	52.8	
Countryside	193	52.3	37.3	47.2	15.5	
Socioeconomic class						**.001[c]**
A–B	63	17.1	30.2	19.0	50.8	
C	205	55.7	35.6	31.7	32.7	
D–E	100	27.2	30.0	46.0	24.0	
Use of medications						**<.001[c]**
No	207	56.1	2.4	49.8	47.8	
Yes	162	43.9	12.4	72.8	14.8	
Kidney disease						.776[d]
No	366	99.2	33.1	33.3	33.6	
Yes	3	0.8	50	50	0	
Bone disease						.109[d]
No	366	99.2	33.6	33.6	32.8	
Yes	3	0.8	33.3	33.3	33.3	
Metabolic disease						>.999[d]
No	365	98.9	33.4	33.4	35.2	
Yes	4	1.1	25	25	50	
Periodic visits to dentist						**<.001[c]**
No	224	60.7	17.0	45.1	37.9	
Yes	145	39.3	58.6	15.2	26.2	

SCA, Sickle cell anaemia; SCT, Sickle cell trait.
Bold denotes statistical significance.
[a]100% in the column.
[b]100% across the row.
[c]Chi-square test.
[d]Fisher's exact test.

associated with root changes and changes in shape, size and position of the teeth. SCA had a higher prevalence of changes in trabecular bone of the maxilla and mandible. Partial loss of LD and changes in trabecular bone of the mandible were more prevalent in SCT subjects than in the controls. The number of teeth with partial loss and LD thickening was higher in the SCA and SCT groups than in the controls (Table 3).

After adjusting for the covariates gender, age, economic class and use of drugs, analyses considering binary outcomes were only able to identify differences between SCA and controls for the external

TABLE 2 Prevalence of dental and bone changes by group

	Total (369)	Control (123)			SCA (123)			SCT (123)			
	N	*n*	%	95% CI	*n*	%	95% CI	*n*	%	95% CI	*p*-value
Dental changes											
Pulp chamber	81	26	21.14	14.30–29.42	29	23.58	16.39–32.07	26	21.14	14.30–29.42	.867[a]
Root	44	12	9.76	5.14–16.42	15	12.20	6.99–19.32	17	13.72	8.26–21.20	.612[a]
Tooth shape	4	2	1.63	0.20–5.75	0	0.00	0–2.95[b]	2	1.63	0.20–5.75	.552[b]
Number of teeth	4	2	1.63	0.20–5.75	0	0.00	0–2.95[b]	2	1.63	0.20–5.75	.552[b]
Tooth size	6	2	1.63	0.20–5.75	1	0.81	0.02–4.45	3	2.44	0.51–6.96	.875[b]
Tooth position	4	2	1.63	0.20–5.75	0	0.00	0–2.95[b]	2	1.63	0.20–5.75	.552[b]
Periapex	13	4	3.25	0.89–8.12	5	4.07	1.33–9.23	4	3.25	0.89–8.12	>.999[b]
Bone changes											
Partial loss of LD	154	40	32.52	24.35–41.55	49	39.84	31.12–49.05	65	52.85	43.64–61.91	**.005**[a]
Total loss of LD	46	12	9.76	5.14–16.42	18	14.63	8.91–22.13	16	13.01	7.62–20.26	.499[a]
LD thickening	40	8	6.50	2.85–12.41	15	12.20	6.99–19.32	17	13.82	8.26–21.20	.153[a]
Trabecular bone of the maxilla	124	17	14.7	8.26–21.20	84	69.4	59.29–76.39	23	19.0	12.24–26.72	**<.001**[a]
Trabecular bone of the mandible	154	19	15.5	9.56–23.07	96	78.1	69.69–85.01	39	31.7	23.61–40.71	**<.001**[a]

SCA, Sickle cell anaemia; SCT, Sickle cell trait; LD, lamina dura; 95% CI, 95% confidence interval.
Bold denotes statistical significance.
[a]Chi-square test.
[b]Fisher's exact test.

resorption (PR: 8.31; CI: 1.21–57.05) and changes in trabecular bone of maxilla (PR: 6.45; CI: 2.75–15.10) and mandible (PR: 5.34; CI: 2.38–11.99). Differences between SCT and controls for partial loss of LD (PR: 1.26; CI: 1.02–1.55), LD thickening (PR: 1.57; CI: 1.01–2.44) and changes in trabecular bone of mandible (PR: 1.43; CI: 1.08–4.83) were also observed (Table 4).

When outcomes were considered as quantitative variables, SCA patients exhibited a greater number of teeth with pulp canal calcification (PR: 1.78; 95% CI: 1.81–76.84) (Figure 1a) and root changes (PR: 3.61; 95% CI: 1.45–8.95), specifically external resorption (PR: 13.40; 95% CI: 3.64–49.41) (Figure 1b). Subjects with SCT had a high number of teeth with root changes (PR: 2:41; 95% CI: 1:36–4:29), specifically hypercementosis (PR: 2.93; 95% CI: 1.25–6.90) (Figure 2a), changes in tooth shape (PR: 1:51; 95% CI: 2.31–57.32), size (PR: 4.90; 95% CI: 1:30–18:52) (Figure 2b) and periapex (PR: 3.17; 95% CI: 1:06–9:50) (Figure 2c) (Table 4).

After adjustment, trabecular bone changes of the maxilla were 2.64 times more prevalent in patients with SCA than in the controls (OR: 2.64; 95% CI: 6.90–61.67). Trabecular bone changes of the mandible were 21.17 times more prevalent in patients with SCA (RP: 21.17; 95% CI: 7.73–57.96) (Figure 1c) and 2.54 times more prevalent in patients with SCT (PR 2.54; 95% CI: 1.34–4.81) than in the controls (Figure 2d). Partial and total loss of the LD were also more prevalent in patients with SCA (Figure 1d) and SCT, and thickening of the LD was seen more frequently in people with SCT (PR: 2.23; 95% CI: 1.37–3.64) than the controls (Figure 2c) (Table 4).

4 | DISCUSSION

Sickle cell anaemia (SCA) and SCT are not associated with higher prevalence of dental abnormalities; however, within individuals with dental abnormalities, homozygous and heterozygous subjects show more affected teeth than the remainder. The results of this study show that SCA is associated with higher number of teeth presenting changes in the pulp chamber (calcification) and root (external resorption). Pulp changes are usually reported with respect to SCA (Costa et al., 2013; Demirbaş Kaya, Aktener, & Unsal, 2004), and pulp canal calcifications were identified previously (Bishop et al., 1995; Taylor et al., 1995). These changes are characterized by the presence of calcified masses in the dental pulp of healthy, diseased and even impacted teeth (Sener, Cobankara, & Akgünlü, 2009). Although the aetiological factors of pulpal calcifications are not well understood, age, gender and systemic and genetic diseases have been implicated (Sener et al., 2009). Pulpal calcifications in individuals with SCA are considered to be the result of an accumulation of sickled cells that cause thrombosis of blood vessels feeding the affected area (Sams, Thornton, & Amamoo, 1990).

Root changes, especially external resorption, are not oral manifestations previously described in patients with SCA. External resorption initiates in the periodontal structure and affects the lateral external surfaces of a tooth (Fernandes, de Ataide, & Wagle, 2013). This sequela is a common one after traumatic injuries, orthodontic movement or chronic infections of periodontal and endodontic structures (Fuss,

TABLE 3 Unadjusted association between SCA and SCT with dental and bone changes compared with the control group

	Unadjusted association							
	Binary outcome (yes or not)				Discrete outcome (number of abnormalities per affected individual)			
	SCA		SCT		SCA		SCT	
Dental changes	PR	95% CI	PR	95% CI	PR	95% CI	PR	95% CI
Pulp chamber	1.11	0.66–1.89	1.00	0.76–1.31	1.11	0.89–1.40	0.97	0.77–1.22
Atresia	0.54	0.21–1.35	0.88	0.58–1.32	**0.42**	**0.22–0.80**	0.71	0.41–1.22
Calcification	2.33	0.60–9.02	1.53	0.78–3.01	3.00	0.81–11.08	3.33	0.92–12.11
Nodules	1.14	0.41–3.15	1.13	0.69–1.86	0.81	0.39–1.69	1.12	0.57–2.21
Taurodontism	2.00	0.86–4.67	0.87	0.51–1.47	1.50	0.80–2.82	0.62	0.28–1.38
Root	1.25	0.59–2.67	1.19	0.82–1.72	1.61	0.89–2.90	**2.17**	**1.24–1.79**
Hypercementosis	0.44	0.14–1.44	0.88	0.54–1.45	0.80	0.31–2.03	1.70	0.78–3.71
External reabsorption	2.75	0.88–8.64	1.41	0.78–2.58	**2.86**	**1.21–6.76**	1.71	0.67–4.35
Tooth form	–	–	1.00	0.38–2.66	–	–	**4.00**	**1.13–14.17**
Number of teeth	–	–	1.00	0.38–2.66	–	–	3.33	0.92–12.11
Agenesis/Anodontia	–	–	1.00	0.25–4.00	–	–	1.00	0.06–15.99
Tooth size	0.50	0.05–5.51	1.22	0.50–2.99	0.67	0.11–4.00	**4.67**	**1.34–16.24**
Microdontia	0.50	0.05–5.51	1.00	0.38–2.66	1.00	0.14–7.10	2.50	0.48–12.88
Tooth position	–	–	1.00	0.38–2.66	–	–	**3.66**	**1.02–13.14**
Giroversion	–	–	0.50	0.17–1.50	0.50	0.09–2.73	0.50	0.09–2.73
Periapex	1.25	0.34–4.65	1.00	0.50–2.00	1.60	0.52–4.89	2.60	0.93–7.29
Radiolucent with LD	1.33	0.30–5.96	1.00	0.45–2.23	2.33	0.60–9.02	1.33	0.30–5.96
Radiolucent without LD	0.50	0.05–5.51	–	–	0.50	0.04–5.51	–	–
Bone changes								
Partial loss of LD	1.23	0.81–1.86	**1.27**	**1.05–1.55**	**1.60**	**1.32–1.92**	**1.92**	**1.60–2.30**
Total loss of LD	1.50	0.72–3.11	1.15	0.79–1.68	1.10	0.80–1.51	1.30	0.96–1.77
LD thickening	1.88	0.79–4.42	1.46	0.96–2.22	**2.12**	**1.33–3.37**	**1.92**	**1.20–3.09**
Trabecular bone of the maxilla	**4.74**	**2.81–7.98**	1.14	0.83–1.56	–	–	–	–
Trabecular bone of the mandible	**5.05**	**3.09–8.26**	**1.43**	**1.09–1.88**	–	–	–	–

SCA, Sickle cell anaemia; SCT, Sickle cell trait; PR, Prevalence ratio; 95% CI, 95% confidence interval; LD, lamina dura.
Bold denotes statistical significance.
– Not evaluated.

Tsesis, & Lin, 2003). The course of tooth resorption involves a complex interaction among inflammatory cells, resorbing cells (odontoclasts) and hard tissue structures (Ne, Witherspoon, & Gutmann, 1999). The plausibility of this finding is suggested to be related to the greater propensity to infections of people with SCA, thus favouring inflammatory mechanisms associated with injury of dental tissues due to biochemical mechanisms.

This study also found that a higher number of teeth presenting hypercementosis and changes in shape, size and periapex were common in people with SCT in the adjusted model. Hypercementosis is a change commonly described in patients with SCA (Soni, 1966; Taylor et al., 1995), but no reports of this event in individuals with SCT have been made. Hypercementosis is characterized by the excessive deposition of non-neoplasic cementum continuous to the normal radicular cementum, thus giving the root a bulky appearance. The "enhanced" root is then surrounded by a radiolucent periodontal ligament space and the adjacent LD is intact (Shoor, Sujir, Mutalik, & Pai, 2014). Our data also showed a prevalence of hypercementosis 2.9 times higher in SCA than in controls, however with no significant differences, probably due to a sample size with low power. Another possible explanation is related to the fact that homozygous individuals are followed by the Brazilian Health System (SUS) since birth, but heterozygous individuals are not. Individuals with SCA could be protected from oral complications caused by vaso-occlusive events because of haematologic treatment, and thus, the observed associations might have been underestimated. This treatment is not provided by SCT individuals.

TABLE 4 Adjusted association between SCA and SCT with dental and bone changes compared with the control group

	Adjusted association[a]							
	Binary outcome (yes or not)				Discrete outcome (number of abnormalities per affected individual)			
	SCA		SCT		SCA		SCT	
Dental changes	PR	95% CI	PR	95% CI	PR	95% CI	PR	95% CI
Pulp chamber	1.05	0.39–2.83	1.02	0.76–1.35	0.79	0.56–1.12	1.04	0.82–1.32
Atresia	2.29	0.45–11.62	0.93	0.60–1.44	1.06	0.38–2.90	0.78	0.44–1.37
Calcification	5.69	0.60–53.53	1.56	0.75–3.22	**1.78**	**1.81–76.84**	3.32	0.88–12.46
Nodules	0.74	0.14–3.88	1.15	0.68–1.96	0.50	0.18–1.37	1.12	0.56–2.27
Taurodontism	2.11	0.42–10.76	1.04	0.59–1.86	1.02	0.30–3.43	0.90	0.39–2.08
Root	2.89	0.74–11.32	1.23	0.83–1.83	**3.61**	**1.45–8.95**	**2.41**	**1.36–4.29**
Hypercementosis	1.23	0.20–7.48	1.00	0.58–1.73	2.90	0.83–1.17	**2.93**	**1.25–6.90**
External reabsorption	**8.31**	**1.21–57.05**	1.35	0.72–2.52	**13.40**	**3.64–49.41**	1.95	0.75–5.06
Tooth form	–	–	1.08	0.38–3.07	–	–	**1.51**	**2.31–57.32**
Number of teeth	–	–	0.79	0.29–2.14	–	–	2.53	0.92–13.50
Agenesis/Anodontia	–	–	0.64	0.16–2.60	–	–	0.42	0.02–6.78
Tooth size	3.72	0.05–29.17	1.04	0.41–2.61	0.29	0.02–3.30	**4.90**	**1.30–18.52**
Microdontia	3.72	0.05–29.17	0.89	0.31–2.55	1.10	0.06–2.16	1.95	0.36–1.68
Tooth position	–	–	0.82	0.30–2.25	–	–	3.47	0.92–13.09
Giroversion	1.82	0.05–62.25	0.47	0.15–1.46	2.81	0.12–63.53	0.43	0.07–2.52
Periapex	1.63	0.17–15.95	0.98	0.48–2.01	1.31	0.22–7.61	**3.17**	**1.06–9.50**
Radiolucent with LD	2.65	0.23–30.49	1.08	0.46–2.52	6.18	0.74–51.26	2.00	0.42–9.46
Radiolucent without LD	0.89	0.01–35.09	–	–	0.99	0.002–495.17	–	–
Bone changes								
Partial loss of LD	1.47	0.70–3.12	**1.06**	**1.02–1.55**	**2.58**	**1.94–2.38**	**1.98**	**1.64–2.38**
Total loss of LD	3.44	0.99–11.94	1.21	0.81–1.81	**2.69**	**1.68–4.31**	**1.65**	**1.19–2.30**
LD thickening	0.64	0.15–2.68	**1.57**	**1.01–2.44**	1.30	0.65–2.61	**2.23**	**1.37–3.64**
Trabecular bone of the maxilla	**6.45**	**2.75–15.10**	1.18	0.85–1.63	–	–	–	–
Trabecular bone of the mandible	**5.34**	**2.38–11.99**	**1.43**	**1.08–4.83**	–	–	–	–

SCA, Sickle cell anaemia; SCT, Sickle cell trait; PR, Prevalence ratio; 95% CI, 95% confidence interval; LD, lamina dura.
Bold denotes statistical significance.
[a]Adjusted for gender, age, economic class and use of drugs; – Not evaluated.

Similarly, no precedent can be found in the literature on the association between SCT and changes in tooth shape, size and periapex. Although this condition is considered asymptomatic, SCT may be a risk factor for adverse events in certain situations (de Carvalho et al., 2015; Tsaras et al., 2009). Evidence shows that individuals with SCT exposed to low oxygen conditions can exhibit changes linked to vaso-occlusive events similar to those described in SCA (Goldsmith et al., 2012; Key & Derebail, 2010), thus increasing the occurrence of dental and bone changes. Among the most common dental abnormalities in individuals homozygous for the Hb S gene are delayed tooth eruption and hypomaturation or hypomineralization of the enamel and dentin (Acharya, 2015; Soni, 1966). A case of hypodontia in an adolescent with the Hb SC genotype, a variant of sickle cell disease, was reported in the literature (Oredugba, 2005). The association between changes in the size and shape of the teeth and SCT may be related to development problems originating from the enamel and dentin as described above.

Although these findings point to an association between exposures (SCA and SCT) and dental changes, it is reasonable to consider that abnormalities of the teeth can be influenced by heredity, genetic and environmental factors (Neville, Damm, Allen, & Chi, 2015d). In this study, data related to the presence of systemic conditions were obtained by interviews, which could represent a measurement bias. Nevertheless, dental changes as hypomaturation or hypomineralization of enamel and dentin, pulp stones, asymptomatic pulp necrosis and hypercementosis are the most commonly described findings in the oral cavity, which are non-pathognomonic but may be characteristic

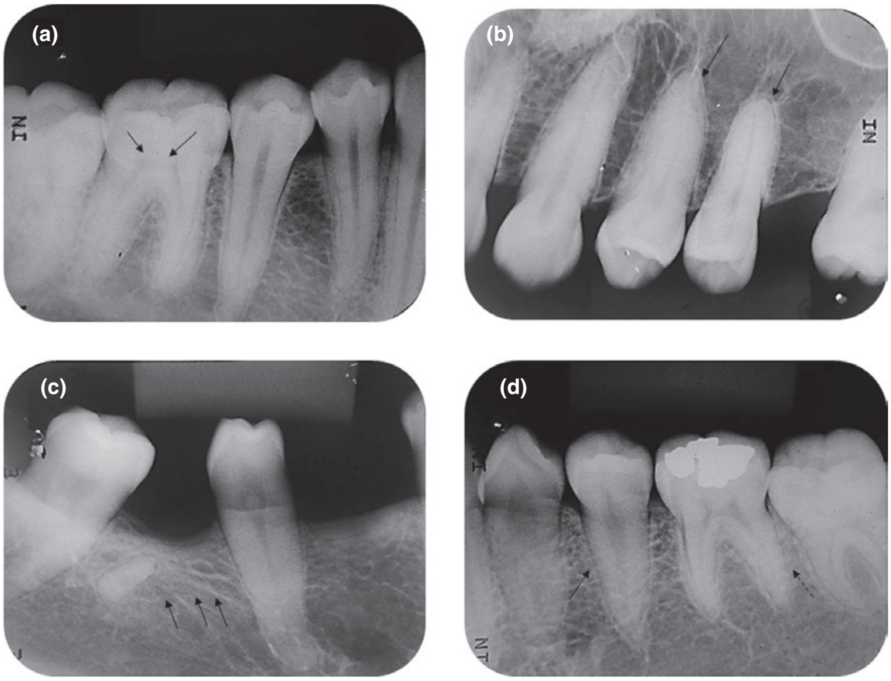

FIGURE 1 Dental and jaw bone changes in subjects with sickle cell anaemia (SCA). Pulp calcification (arrows) in the first molar (a). External root resorption (arrows) in the first and second premolars (b). Trabecular bone change of the mandible (spider web pattern) in the premolar and molar areas (arrows) (c). Partial (arrow) and total (dotted arrow) absence of lamina dura in mesial root of second premolar and in distal root of first molar, respectively (d)

of the disease (Costa et al., 2012; da Fonseca, Oueis, & Casamassimo, 2007).

Our findings suggest that jaw bone changes were associated with SCA and SCT, even after adjusting for covariates, as age. It was observed that bone changes evidenced in the jaws are more notable in children. It is known that bone involvement is the commonest clinical manifestation of sickle cell haemoglobinopathies both in the acute setting such as painful vaso-occlusive crises, and as a source of chronic and progressive disability such as avascular necrosis (Almeida & Roberts, 2005). In children with SCA under the age of 7 years, particularly those aged 1–2 years, vaso-occlusive crises frequently occur in the small bone of the hands and feet. In buccal cavity, it is evidenced by maxillary prominence and malocclusion and has been described as the main jaw abnormality seen in SCA patients (Almeida & Roberts, 2005; Kim & Miller, 2002). Oredugba and Savage (2002) observed that SCA subjects aged 1–18 years had higher prevalence of maxillary protrusion and Class II malocclusion that controls, which show that these changes can affect younger individuals.

In the adjusted model, SCA was associated with jaw bone changes and alterations in LD. Previous studies indicated that these radiographic findings are commonly related to the disease. These findings can be related to erythroblastic hyperplasia and compensatory medullary expansion due to vaso-occlusion and are a source of chronic progressive disability such as avascular necrosis (Almeida & Roberts, 2005). To confirm these observations, Franco, Gonçalves, and dos Santos (2007) evaluated eight patients with SCA using periapical radiographs and found that all of them had similar bone changes with a "step-ladder" trabecular pattern. Demirbaş, Ergün, Güneri, Aktener, and Boyacioğlu (2008) reported that 67% of 36 patients with SCA presented with a reduced trabecular bone density and increased medullary spaces, and 28% of the sample presented with the "step-ladder" trabecular pattern. In evaluating 71 patients with SCA, Neves et al. (2012) found statistically significant associations between history of clinical jaundice and increased trabecular spacing ($p = .02$) and between a history of stroke and a horizontal pattern of trabecular bone ($p = .04$). These findings demonstrate the importance of studies on the oral complications of the disease as predictors of systemic diseases.

Similar to SCA, SCT was associated with changes in the LD and trabecular bone of the mandible. These bone changes confirm the findings of Halstead (1970), who found that some heterozygous individuals (SCT) had abnormal bone development when assessing the radiographic aspects of oral manifestations of haemoglobinopathies. In a case report of a patient with SCT, Gillis and West (2004) described obvious changes in patterns of trabecular bone similar to those seen in individuals with SCA; these changes were a result of increased haematopoiesis occurring in the medullary spaces. The increase in trabecular space can lead to a reduction in bone density, thus making it more brittle and possibly more susceptible to bone defects.

In the current study, some outcomes were weakly or not associated with SCA and/or SCT, especially dental abnormalities. Changes in tooth size, shape, and structure result from disturbances during the morphodifferentiation stage of development (Altug-Atac, Erdem, Tyndall, & Wright, 2007). Several studies evaluated dental anomalies in various non-syndromic populations, but their results are conflicting (Bailit, 1975; Clayton, 1957; al-Emran, 1990; Hamasha & Al-Khateeb, 2004; Järvinen & Lehtinen, 1981; Ooshima, Ishida, Mishima, & Sobue, 1996; Shah, Boyd, & Vakil, 1978; Thongudomporn & Freer, 1998). Yassin (2016) investigated the distribution and association of dental anomalies among Saudi children and observed that dental anomalies are not a rare finding during routine dental examination. In the other hand, Ledesma-Montes, Garcés-Ortíz, Salcido-García, and Hernández-Flores (2016) verified that 179 (5.1%) of the 3,522 patients reviewed harboured developmental dental alterations. This finding suggest that some dental abnormalities have low frequencies in general

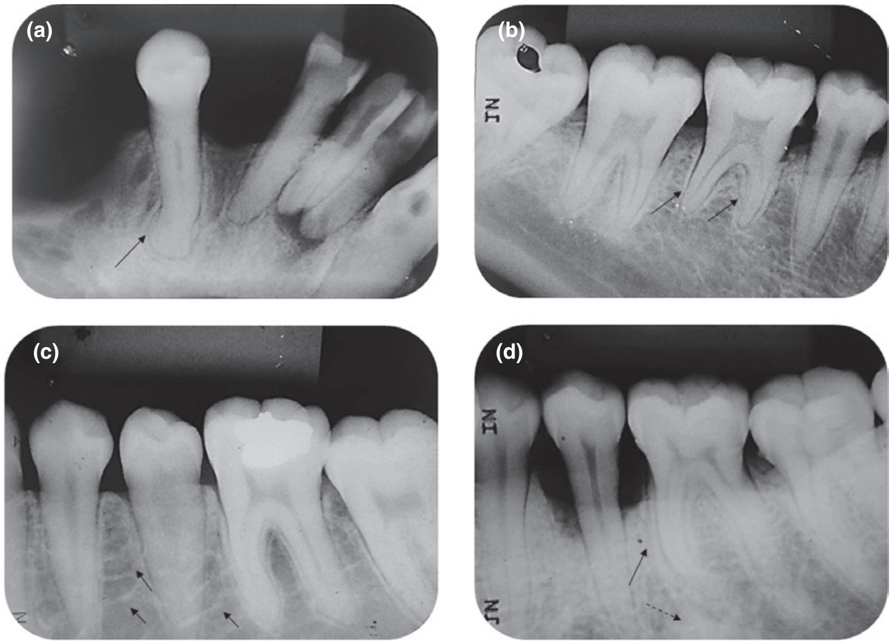

FIGURE 2 Dental and jaw bone changes in subjects with sickle cell trait (SCT). Hypercementosis (arrow) in the first premolar (a). Changes in tooth shape and size (arrow) in the first molar (b). Increased thickness (arrow) and radiopaque periapex change (dotted arrow) in mesial root of first molar (c). Trabecular bone change of the mandible (step-ladder pattern) in the premolar and molar areas (arrows) (d)

populations, which was also observed in the sample of this study. It is suggested that our sample size did not have sufficient power to detect these differences between groups, despite being the study with the largest sample size identified to date.

Nevertheless, care must be taken with the external validity of our findings, especially in cases where associations have not been evidenced, despite high prevalence ratios. However, to identify associations for the different outcomes of the study—considering the power of 80%, alpha of 5% and ratio of 1:1 between exposed and non-exposed—it would be required groups with a minimum sample size ranging from 2,658 (for root changes) up to 8,473 (for periapex changes) subjects in each group. This could make studies with SCA and SCT groups unfeasible.

Despite this limitation, the sample size had sufficient power to detect some differences between groups, especially for variables related to bone changes. SCA and SCT subjects have higher frequencies of trabecular bone changes. Some studies usually report that some radiographic features of SCA are increased radiolucency of the jaws due to the decreased number of trabeculae, coarse trabeculae pattern and step-ladder appearance created in the interdental alveolar bone by horizontal rows of trabeculation (da Fonseca et al., 2007; Neves et al., 2012), but only two studies reported theses changes in SCT subjects (Gillis & West, 2004; Halstead, 1970). The presence of these radiographic features can be deterministic for the existence of SCA and SCT.

The periapical radiographic imaging technique was used because it can show possible dental and bone changes that are not seen clinically. Compared with panoramic radiographs, periapical radiographs are more accurate with less distortion and greater detail (Kim, Obst, Zehaczek, & Geenen, 2008), thereby providing precision in the assessment of the current study. In this study, visual analysis by three blinded and calibrated examiners identified significant differences among the groups. In a similar study, the bone patterns of individuals with and without SCA were evaluated by periapical radiographs analysed by software (White, Cohen, & Mourshed, 2000). Patients with SCA were found to have significantly larger medullary spaces and a less complex trabecular structure than the control group, consistent with the findings of this study.

A limitation of retrospective studies is the possibility of recall bias (Sutherland, 2001). However, evidence shows that this bias is low in groups of people undergoing treatment for chronic diseases (Colditz, 2010). Moreover, the frequencies of changes were obtained by evaluating images, and thus, they are not subject to such a bias. One limitation concerns to the assessment of oral complications in individuals at only in one point in time. This situation prevents the diagnosis of changes already addressed and resolved, and the effect of SCA and SCT on the investigated changes may have been underestimated. Moreover, the most severely ill patients and those who died might have had a higher risk of developing dental problems, but they were not included in this research.

Errors in measuring exposure or disease can be an important source of bias, especially in epidemiological studies (Coggon, Rose, & Barker, 2003). To reduce measurement bias in this investigation, evaluations were conducted by three matched blind examiners, who were trained during 2 months. Kappa values for inter- and intra-examiner agreement for detection of dental and bone changes exhibited moderate, substantial and very good concordances in accordance with Cohen's kappa concordance index (Landis & Koch, 1977). To provide any inference based on reproducibility and reliability of diagnoses, golden standards for each outcome were considered. However, racial differences, sampling techniques and different diagnostic criteria can influence the results (Altug-Atac et al., 2007) and be considered a limitation related to evaluations of maxillofacial abnormalities.

To date, no study has reported the association of SCA and SCT with bone and dental changes, especially using multivariate analysis. Only one article reported a correlation between clinical severity of SCA and frequency of bone changes (Neves et al., 2012), but this study had methodological limitations because of the small sample size and the lack of a control group. Note that the sample size in this study was calculated and had sufficient power to report statistical differences accurately among groups for many associations. In addition, the outcomes were classified in two ways, which reflect different aspects of the abnormalities: their prevalence (binary) and the intensity of their occurrence (count). Considering outcome as the number of abnormalities per affected individual increases the power of the sample to identify differences between groups. All associations identified when considering binary outcomes were also verified in the analyses with quantitative outcomes, but the opposite did not occur. Still, we decided to keep both ratings.

5 | CONCLUSIONS

- The most frequent dental alterations in SCA patients in the present sample were pulp calcification and external resorption of the root.
- Trabecular bone changes of the maxilla more prevalent in individuals with SCA.
- SCT subjects had higher frequency of root changes, such as hypercementosis, changes in the shape, size and periapex.
- Thickening of the LD was seen more frequently in people with SCT.
- Trabecular bone changes of the mandible, partial and total loss of LD were most prevalent in both homozygous and heterozygous individuals.

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CONFLICT OF INTERESTS

None to declare.

AUTHOR CONTRIBUTIONS

Soraia de Fátima Carvalho Souza supervised the study, analysed the data and revised the manuscript. Halinna Larissa Cruz Correia de Carvalho analysed the data and drafted the manuscript. Cyrene Piazera Silva Costa revised the manuscript. Erika Bárbara Abreu Fonseca Thomaz analysed and interpreted the data and revised the manuscript.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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