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Title

Orthodontic pain trajectories in adolescents: Exploring the between- and within-subject variability in pain perception

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Author Contributions

Satpal S Sandhu developed the study concept and design including the sample size estimation, conducted the study and was responsible for data collection. George Leckie was involved with the statistical analysis and presentation of results. Satpal S Sandhu drafted the initial version of the manuscript, which was revised by both the authors. Both authors approved the final version of the manuscript for submission.

Orthodontic pain trajectories in adolescents: Exploring the between- and within-subject variability in pain perception

Abstract

Introduction: The objective of this study was to assess the effect of age, sex and age-sex interaction effects on mean pain trajectories and individual variation in the pain experienced by adolescents after orthodontic separator placement.

Material and methods: 115 subjects (mean age 14.99, SD ±1.90; males 56, 48.7%; females 59, 51.3%) were included in this study. Orthodontic separators were placed in the mesial and distal contact point of maxillary and mandibular first molars. A 100 mm Visual Analogue Scale (VAS) was used for pain assessment over 11 pre-specified time points: 1 hour, 2 hours, 4 hours, 12 hours, 24 hours, 36 hours, 48 hours, 72 hours, 96 hours, 120 hours, 144 hours. A "mixed-effects location scale model" was used for the data analysis to directly model between-subject (BS) and within-subject (WS) variability in pain in addition to the usual modelling of mean pain as a function of age, sex and time.

Results: Mean initial pain after 1 hour of separator placement for 12-15 year male group was 13.52 mm on VAS scale, which initially increased rapidly (linear estimate 9.16; p 0.000; 95% CI -8.65 to 9.67), but decelerated with time (quadratic estimate -0.95; p 0.000; 95% CI -1.0 to -0.90), suggesting an inverted 'U' shaped mean pain trajectory. Age, sex and age-sex interaction effects did not significantly influence initial pain. Compared to 12-15 year male group, 15-18 year female group reported the steepest rise in the pain (estimate 8.55; p 0.00; 95% CI 7.40 to 9.70), and as a result, experienced the most overall pain. 12-15 year male group reported minimum BS variation (SD ±4.6 mm) as well as the WS variation (SD ±5.5 mm). The BS variation was highest for the 12-15 year female group (SD ±9.8 mm) whereas the WS variation was highest for the 15-18 year female group (SD ±10.1 mm).

Conclusion: 12-15 year males reported the least mean average pain intensity as well as the minimum subjective variation in terms of BS variance and WS variance. 15-18 year females experienced maximum mean pain intensity as well as the highest daily fluctuation in pain intensity. 12-15 year females were most different from one another in terms of their overall pain experience.

Introduction

Orthodontic force application during tooth movement induces complex biological response in and around the periodontium resulting in release of inflammatory mediators such as prostaglandin-E_2 (PGE_2), interleukin 1-beta (IL-1β) and substance P (SP). These substances, which are essential for bone remodelling during tooth movement, also result in pain.[1,2]

Pain is both patient and time dependent resulting in substantial heterogeneity in patients' reported pain trajectories over time.[3] Put differently, pain is both a between-subject (BS) and within-subject (WS) phenomenon. Evidence shows that most orthodontic patients report that pain commences during the first couple of hours of orthodontic force application, reaches peak intensity level after one day, and then eventually declines to normal levels after 7 days.[4-7]

Bergius et al[5,6] report that the experience of pain varied substantially among subjects after elastic separators placement, suggesting BS variation in orthodontic pain perception. The authors further report that patient gender had a significant influence on orthodontic pain perception. A recent study highlighted the fact that patients' age and sex also have strong interaction as well as direct effects on orthodontic pain perception.[4]

Describing pain trajectories would improve understanding of how orthodontic pain conditions develop over time; and whether individuals differ in pain perception. This understanding would then enable better management of orthodontic pain. In orthodontics, no study has ever been undertaken in this direction to understand pain trajectories. Importantly, previous studies have largely ignored BS and WS variation of pain and how these distinct sources of variation may themselves depend on patients' characteristics. For example, do younger subjects tend to vary more in the overall average pain they experience (i.e., BS variation) than older subjects? Do females tend to report more fluctuating (i.e., erratic or volatile) pain trajectories (i.e., WS variation) than males?

Mixed-effects models, also known as multilevel models and hierarchical linear models, can be used to analyse the evolution of subjects' individual outcome trajectories over time and to relate variation in these trajectories to subjects' time-invariant characteristics.[8,9] Mixed-effects models can also incorporate time-varying subject characteristics to model occasion-to-occasion deflections to or departures from subjects' trajectories. Thus, mixed-effects models provide a popular way to not only estimate overall mean relationships, but to additionally quantify and then explain the degree of BS and WS variation in individuals' outcomes over time.[8,9]

Recently, Hedeker et al.[10,11] extended the standard two-level random-intercept mixed-effects model to additionally model as a function of the covariates both the BS variation in subjects' trajectories about their overall mean trajectory and the WS variation in their observed measurements about their own trajectories. They term their model the "mixed-effects location scale model" where "location" refers to the usual modelling of the mean response, while "scale" refers to the new direct modelling of the BS and WS response variability. They implement their model in the stand-alone program MIXREGLS.[11]

The objective of this clinical research work was to evaluate the overall mean orthodontic pain trajectory and the BS and WS variation about this over a week's time period following orthodontic separator placement; and to examine the influence of age, sex and age-sex interaction effects on the overall mean, and BS and WS variances using mixed-effects location scale models.

Material and methods

Sample size estimation

Sample size calculation was based on a power analysis concept used in a recent study where the authors investigated the age-sex interaction effect on mean average orthodontic pain perception.[4] Briefly, in this approach, which is based on the power analysis for a 2 x 2 factorial

design,[12] sample size is estimated for either of the binary coded groups (e.g. sex or dichotomized age) assuming that interest lies in detecting the same effect size for each binary group, and then doubling the estimated sample size to detect the interaction effect.[4,12]

The parameter estimates (including time function regression coefficients, and BS and WS variance etc.) required for the power analysis were obtained from the authors of the previous study.[4] Based on these parameters, power analysis for the quadratic trend analysis was undertaken to determine the sample size, as recommended for the mixed-effect model for binary coded groups (e.g. male and female).[13] The standardized Cohen's d effect size for a mixed-effects analysis is defined as d= slope coefficient /√(BS variance + WS variance), where the slope coefficient may be for any polynomial function of time such as linear, quadratic etc.[13]

Power analysis based on a study design with one baseline and 10 follow-up repeated measurements per subjects, an attrition rate of 10%, a moderate effect size (Cohen's d=0.5) for the difference in slopes among the groups at a significance level of 0.05 and a power level of 0.80, revealed that 60 participants (30 in each group) were required. Therefore, the total sample size required to detect the age-sex interaction effect on the mean response was 120 subjects (30 in each of the four groups).

The Cohen's medium effect size for mean difference (d=0.5) value corresponds to the Cohen's medium effect size for correlation (r=.30) which can be used to find matching values for the regression coefficients in terms of 9% (R^2=.09) variance explained, which could be rounded off to approximately 10%.[14] Therefore, sample size in this study was also calculated to be sufficient to detect a 10% difference in the variance among the groups.

Participants

The participants were consecutive patients who visited the private office of the first author for orthodontic treatment, and were enrolled in the study if all the inclusion criteria were satisfied and informed consent could be obtained. In total, 120 orthodontic patients were included in this study. Study protocol was approved by the local Ethical Review Committee.

The inclusion criteria were: (1) 12-18 year-old males and females who required fixed orthodontic treatment, (2) presence of erupted permanent first and second molars and absence of posterior open bite and interdental spaces, (3) no concurrent use of any anti-inflammatory drugs, (4) caries-free dentition with healthy periodontium, (5) voluntary participation in the study confirmed by signing the informed consent form. The exclusion criteria were: (1) medical condition / systemic diseases (e.g. epilepsy, juvenile diabetes etc.) that precluded the use of prospective fixed orthodontic appliance, (2) participants having any chronic pain or orofacial region/dental pain.

Procedure and outcome assessment

A previously established and standardized research model was used to assess orthodontic pain perception.[5,6] In this model, orthodontic elastic separators are placed bilaterally, mesial and distal of the first molars in at least one jaw in adolescents (12-18 year male and female orthodontic patients) and then pain intensity is assessed over a one week time period by using a 100 mm Visual Analogue Scale.

In our study, orthodontic elastic separators (3M Unitek, calif.) were placed in the mesial and distal contact point of both maxillary and mandibular first molars. For all participants, separators were placed in the evening, between 5pm to 7pm, though on different days. This was done to ensure that for all participants, pain assessment time would be the same to

minimize the influence of natural diurnal variation in the pain intensity level. On the day of separator placement, booklets comprised of the pain assessment scale and written instructions were provided to the participants.

Pain was assessed by using a Visual Analogue Scale (VAS), which is a 100 mm long horizontal line where one end corresponds to "no pain" and the other end indicates "worst pain possible". VAS is a valid and reliable scale for pain assessment.[15] To better understand the orthodontic pain trajectories, especially the WS variance component, we decided to increase the number of pain assessment occasions as compared to the previously established model.[5,6] In our study, the number of occasions for pain assessment was eleven; which is more than double the pain assessment occasions (five) used in previous model.[5,6] Pain was assessed at the following time periods (in hours) after orthodontic separator placement: 1 hour, 2 hours, 4 hours, 12 hours, 24 hours, 36 hours, 48 hours, 72 hours, 96 hours, 120 hours, 144 hours.

To alleviate any confusion regarding the actual time of pain assessment, we reported pain assessment time in hours instead of morning/evening time period of day. For example, since we inserted orthodontic separators in the evening, therefore the day 1 morning would be corresponding to 12 hours of pain assessment in contrast to notion usually practiced in orthodontic literature where it is assumed that day 1 morning represents 24 hour time period of pain assessment.

A trained research assistant, who was blinded to study, was responsible for data collection. VAS score in mm was measured from the left margin of VAS scale to the nearest millimetre using metallic scale. To examine the reproducibility and reliability of VAS score measurements, 30 randomly selected VAS scales were measured by the first author independently. Intra-Class Correlation coefficient of 0.93 showed excellent reproducibility and reliability.

Participants were also asked to record their analgesic consumption at each time point. No specific analgesic was prescribed and participants were free to consume over-the-counter (OTC) analgesics of their choice. Since no restrictions were applied in terms of dose, frequency or type of analgesics, analgesic consumption data will be used only for descriptive purposes and will not be included in the analysis.

Statistical analysis

Orthodontic pain trajectories were estimated using two-level random-intercept mixed-effects location scale models, treating the repeated measurements at level-1 nested within subjects at level-2. The models consist of three separate equations for simultaneous modelling of the mean response, the log of the BS variance, and the log of the WS variance each as a function of the covariates. The log-link is employed to ensure positive BS and WS variances. All models were fitted using the MIXREGLS program,[11] calling it from within Stata (version 13, StataCorp LP, College Station, TX 77845) using the runmixregls command.[16] A complete technical description of the mixed-effects location scale model and its adaption to the current orthodontic pain study, as well as the details of model fit/run-in is described in the online-supplementary-material.

Results

Of the 120 patients included in this study, three patient did not return the questionnaire; and two patients did not report back. The remaining 115 patients (mean age 14.99, SD ±1.90; males 56, 48.7%; females 59, 51.3%) adhered to the study protocol and provided pain data over the observed time period following orthodontic separator placement. An advantage of mixed-effects models for repeated measures data is that they can handle subject-to-subject variation in the timing of measurements as well as the missing data under the missing at random (MAR) assumption, [10,14] and therefore subjects do not need to provide outcome measurements at

exactly the same time points. In our study, subjects were asked to record their pain intensity as close as possible to the scheduled measurement occasions (1, 2, 4, 12 hours etc.). When subjects failed to record pain at a scheduled measurement occasion (e.g. asleep) they would simply record their pain intensity and time of measurement at the first available opportunity. As stated in the methodological section, we intended to collect 11 observations per subject. Results revealed that the average number of observations per subject was 10.5 with a minimum of 9 and a maximum of 11 suggesting that missing data was not a major problem in our study.

The descriptive statistics showing the demographic characteristics and the clinical data at each time point are shown in Table 1. All four groups were well matched for number of subjects as well as the mean age for male and female subjects in the 12-15 year and 15-18 year age groups. The mean pain score data shows that peak pain intensity level plateaued for all four groups between 24 hours to 48 hours and that the mean differences between the four groups were also most pronounced during this period. The frequency of analgesic consumption was also highest at this point. Over the seven day period as a whole, the pain intensity level and frequency of analgesic consumption was highest for 15-18 year female group and lowest for the 12-15 year male group. Individual pain trajectories for each group are shown in Figure 1 and reveal substantial BS heterogeneity in VAS scores: some individuals in general report higher pain across all occasions than other individuals. Figure 1 also reveals substantial WS heterogeneity in VAS scores: subjects' individual pain trajectories are not smooth, rather there is a degree of occasion-to-occasion volatility in subjects' pain profiles.

The results from the mixed-effects location scale analysis are shown in Table 2. The variables included in this final model were based on the best fitting model identified by likelihood ratio tests and the information criterion. The close fit of the mean fitted VAS score trajectories to the mean observed VAS score trajectories further confirmed the good model fit (Figure B1 of online-supplementary-material). The histogram and Q-Q plots also showed no

threat to the random effects normality assumptions (Figure B2 of online-supplementary-material).

Mean average pain trajectory

The model predicted mean pain trajectories for each group are shown as Figure 2. The mean initial pain i.e. 1 hour (time=0) after orthodontic separator placement was 13.52 mm on VAS scale for 12-15 year male group (coding 12-15 age=0; sex=0) and there was no significant main effect of age (estimate -0.61; p 0.728; 95% CI -4.02 to 2.81), sex (estimate 1.80; p 0.363; 95% CI -2.08 to 5.69) or age-sex interaction effect (estimate -0.02; p 0.994; 95% CI -5.40 to 5.36) on initial pain level.

From initial pain onwards, 12-15 year male group showed a statistically significant increase in pain with time (linear estimate 9.16; p 0.000; 95% CI -8.65 to 9.67), but this rate decelerated with time (quadratic estimate -0.95; p 0.000; 95% CI -1.0 to -0.90), suggesting an inverted 'U' shaped mean average pain trajectory (see Figure 2).

Compared to 12-15 year male group, 15-18 year male group (estimate 3.88; p 0.00; 95% CI 3.11 to 4.64) and 12-15 year female group (estimate 4.32; p 0.000; 95% CI 3.42 to 5.21) showed significantly steeper rise in pain. The corresponding Cohen's d effect size can be estimated as the slope coefficient /√ (BS variance + WS variance). The required estimates of BS variance and WS variance are provided in the next two sections of the results.

However, the significant age-sex interaction effect with time (estimate 0.36; p 0.042; 95% CI -0.01 to 0.71) revealed that the difference in the rate of increase in pain between male and female subjects was conditional on age and as a result, the rate of increase in pain was further increased by 0.36 units in 15-18 year female group as compared to the 12-15 year female group. The results for the pairwise comparisons (by using Stata's 'lincom' command)

to detect the difference in the linear rate of increase in pain among the four groups (Table C1 of online-supplementary material) show the greatest difference was between the 15-18 year female and 12-15 year male groups (estimate 8.55; p 0.000; 95% CI 7.40 to 9.70).

Compared to 12-15 year male group, the deceleration was significantly faster for 15-18 year male group (estimate -0.37; p 0.000; 95% CI -0.44 to -0.29) and 12-15 year female group (estimate -0.42; p 0.000; 95% CI -0.51 to -0.34). For the quadratic trend, age-sex interaction effect was not included in the model because it led to convergence difficulties. However, a likely reason for this is that the age and sex interactions with the quadratic trend captured the trajectories appropriately and adequately. Therefore, the deceleration in the rate of increase in pain for females was not conditional on age and females attained higher peak pain intensity in both age groups.

Therefore, compared to the 12-15 year male group, the 15-18 year female group experienced the most rapid increase resulting in highest peak pain intensity level, however, owing to their faster rate of deceleration in pain, the difference in pain intensity decreased towards the end of the study period.

Interestingly, findings reveal a plateau of peak pain intensity level for all four groups between Time '4' (24 hours) to Time '6' (48 hours) with peak around the Time '5' (36 hours). The fact that Time is entered as a polynomial makes it straightforward to predict the actual Time at which each group attained peak level of pain intensity. Full details are provided in the online-supplementary material (equation 16) and estimates were obtained using Stata's 'nlcom' command.

Results showed the times of peak mean pain intensity for the 12-15 year male, 12-15 year female, 15-18 year male, and 15-18 year female groups were 4.83, 4.92, 4.96 and 5.10 on the model's Time scale (Table C2 of online-supplementary material) which implies that the

12-15 year male, 12-15 year female and 15-18 year male groups attained peak mean pain intensity level before 36 hours; whereas the 15-18 year female group reported peak mean pain intensity after 36 hours. The results for pairwise comparisons to detect the difference in the time of peak mean pain intensity level (Table C3 of online-supplementary material) shows that the difference was significant between 15-18 year female group and all other three groups.

Between-subject (BS) variation in pain perception

The BS variance function shows that having adjusted VAS scores for time, covariates and covariates-by-time interaction effects, there was significant remaining variability in terms of subjects' individual trajectories about the overall mean average trajectory. Compared to 12-15 year males, 15-18 year males showed greater BS variation but not significantly so (estimate 0.79; p 0.060; 95% CI -0.03 to 1.61). However, 12-15 year females were significantly more variable than 12-15 year males (estimate 1.53; p 0.000; 95% CI 0.75 to 2.30). Interestingly, though not significant, the age-sex interaction effect was negative for the BS variance estimate implying that 15-18 year females are actually less variable as compared to 12-15 year females.

The predicted BS variance for the 12-15 year male, 12-15 year female, 15-18 year male and 15-18 year female groups were 21.19, 97.47, 44.66, and 84.98 respectively. The standard deviation of BS variation can be calculated from variance as SD=√variance. Further, the model implied 95% range for such variation can be calculated as SD*2*1.96. Therefore, the SD (95% range) for the 12-15 year male, 12-15 year female, 15-18 year male and 15-18 year female groups BS variation are ±4.60 mm (±18.04 mm), ±9.87 mm (±38.70 mm), ±6.65 mm (±26.07 mm), and± 9.21 mm (±36.13 mm) respectively. Thus females exhibited greater individual heterogeneity in pain than males, and this variation was greatest for 12-15 year females.

Within-subject (WS) variation in pain perception

The WS variance function captures the occasion-to-occasion variation in pain intensity as a function of the evolution of time and covariates (age, sex and age-sex effect), and provides insight into each subject's fluctuation in pain intensity around his/her individual mean pain trajectory. We also examined interaction of the covariates with linear and quadratic time terms but the estimates were not significant and there was no improvement in the model fit.

The results show that the degree of WS variation exhibited by the typical 12-15 year male was significantly related to both the linear (estimate 0.55; p 0.000; 95% CI 0.40 to 0.70) and quadratic (estimate -0.06; p 0.000; 95% CI -0.07 to -0.04) components of the time trend. Compared to a typical 12-15 year male, the 15-18 year male showed greater WS variance at all occasions, but this difference was not significant (estimate 0.22; p 0.289; 95% CI -0.19 to 0.63). However, the 12-15 year female group showed significantly more variable daily pain experience (estimate 0.69; p 0.000; 95% CI 0.31 to 1.07) as compared to the 12-15 year male group. Though not significant, it is interesting to note that in contrast to the BS variance, the age-sex interaction effect for the WS variance is positive (estimate 0.30; p 0.270; 95% CI -0.24 to 0.84) which implies that a typical 15-18 year female has greater daily variation in pain as compared to the 12-15 year female.

The predicted population-averaged WS variances for the 12-15 year male, 12-15 year female, 15-18 year male and 15-18 year female groups were 31.96, 63.84, 40.86, and 107.66 respectively. The corresponding SD (95% range) for 12-15 year male, 12-15 year female, 15-18 year male and 15-18 year female groups WS variation are ±5.50 mm (±21.59 mm), ±7.78 mm (±30.51 mm), ±6.22 mm (±24.39 mm), and ±10.11 mm (±39.63 mm) respectively.

In other words, the typical female has a more erratic series of pain measurements about her individual quadratic trajectory than does the typical male; and this variation was greatest for 15-18 year females.

Association between subjects' mean pain levels and their WS variability

The linear random-location effects are positively associated with the WS variance and so, having adjusted for all the covariates, subjects with higher mean pain scores tend to have higher pain variability (estimate 0.25; p 0.001; 95% CI 0.10 to 0.39). Finally, the BS random-scale standard deviation which allows for individual-to-individual heterogeneity in individuals' WS variances remains significant even after adjusting for the time, age and sex of the individual suggesting that there is an unexplained component to the within-subjects' pain variability (estimate 0.56; p 0.001; 95% CI 0.430 to 0.688). Thus some individuals present more erratic series of pain measurements than others and this is not simply explained by their age and gender nor is it simply related to their overall mean level of pain.

Intra-class correlation coefficient (ICC)

Based on the predicted BS and WS variances, the ICC can be calculated for each group. The ICC represents the proportion of overall variation in unexplained pain perception which lies between subjects[11]. It is also the expected residual correlation between two observations from the same subject and therefore quantifies the remaining clustering or dependency in the data.[16] The ICC, derived as the BS variance divided by the sum of the BS variance and WS variance, is calculated for the 12-15 year male, 12-15 year female, 15-18 year male and 15-18 year female groups as 0.42, 0.62, 0.54, and 0.46 respectively. Thus, while there was substantial residual clustering for all four groups, subjects in the 12-15 year female group showed disproportionately high variation in the overall average levels of pain experienced coupled with disproportionately low fluctuation in daily pain perception.

Discussion

In this study, we examined individual pain trajectories as well as BS and WS variation in orthodontic pain perception using the recently proposed mixed-effects location scale model.

Our results support the claims made by recent studies which reported that age and sex of individual has a significant influence on orthodontic pain perception[4]; and that substantial individual variation exists in orthodontic pain perception among adolescents.[2,5,6] However, unlike these previous studies which made generalized claims regarding these effects, we were able to explicitly explore the simultaneous effect of age and sex of individual on all three areas of interest i.e. mean average trajectories, BS variation, and WS variation.

The mean (average) estimates showed that pain started almost immediately (within 1 hour) after orthodontic force application, a finding in agreement with previous studies.[1,2] The observed trend of pain was not linear but followed a non-linear approximately quadratic profile (inverted 'U' shape), supporting the findings of a recent study which claimed that orthodontic pain follows a quadratic trend.[4]

Generally it is claimed that peak orthodontic pain level is reached on day one morning or 24 hours after orthodontic separator placement.[1,2,5,6] However, this claim made by authors of previous studies has an inherent flaw, as rightly pointed out by the authors of a recent study.[7] Since none of the previous studies reported the actual time of force application and the subsequent time of pain assessment, therefore, it is difficult to ascertain the actual time of peak intensity level. After controlling for this factor of variation i.e. time (standardizing time for force application and reporting of pain assessment in hours), our results shows that there is a plateau of peak pain intensity level ranging from 24 hours to 48 hours of orthodontic force application and interestingly, the time taken to reach peak pain intensity level after orthodontic

force application is significantly longer for 15-18 year female (after 36 hours) as compared to the other three groups (before 36 hours).

The observed mean average pain trend perhaps reflects the underlying biological responses to orthodontic force application. Interleukin-1β (IL-1 β), the first mediator to regulate bone remodelling in response to orthodontic force, also plays a significant role in orthodontic pain response by inducing the secretion of pain producing pro-inflammatory mediators.[1] Studies[1,2] have demonstrated that the IL-1 β concentration increases after 1 hour of orthodontic force application; reaches a peak after 24 hours; and subsequently declines to about the normal level after around one week. However, these studies assessed the concentration of IL-1 β at only 24 hours around the plateau of peak pain intensity observed in our study. Perhaps future studies can provide better insight into the biological mediators of pain response by assessing the concentrations of these substances at more frequent intervals around the 24 hours' time period.

Females were associated with higher mean pain perception compared to males. However a significant positive age-sex interaction effect revealed that the effect of sex on pain was mediated by the age of subjects. As a result, 15-18 year female group experienced the most pain whereas the 12-15 year male group reported least mean pain response.

Various bio-physiologic and psychosocial factors can contribute to age and sex differences in pain perception during adolescence.[17,18] Evidence shows that in response to painful stimulus, females have significantly greater activation of the contralateral prefrontal cortex, the contralateral insula and the thalamus compared with males, suggesting an inherent sexual dimorphism in response to pain.[19] Further, the difference for pain perception among male and female subjects changes significantly after puberty/menarche onset (initiation of menstrual cycles) due to complex central/peripheral interactions between pain specific neurotransmitters and ovarian hormones.[18,20]

In our study, the difference in the mean pain perception as well as the BS and WS variance amongst 12-15 year females and 15-18 year females could be possibly explained by the expected difference in the number of females with menarche onset in these two age groups, as pointed out by the authors of recent orthodontic pain study.[4] The median age for menarche onset is 12.43 years and nearly 90% of girls are menstruating by the age of 13.75 years.[21] Therefore, females in the 12-15 year age group were more heterogeneous in terms of menarche onset as compared to those in the 15-18 year age group where almost all females could be expected to have positive menarche onset.

The heterogeneity in number of females with menarche onset in the 12-15 year female group might explain the highest between-subject variation observed for that group if females who achieved menarche onset did indeed experience significantly greater pain as compared to females who had not yet started their menstruation periods. The lower between-subject variation among 15-18 year females shows that almost all older female adolescents behaved similarly in terms of pain perception, perhaps owing to the similar positive status of menarche onset.

In contrast to the between-subject variation, 15-18 year-old females showed the greatest variation in within-subject daily fluctuation of pain. The large WS variance observed for these subjects might again be explained by the greater number of females with positive menarche onset in this group as compared to 12-15 year female group. Evidence suggests that hormonal fluctuation during the menstrual cycle modulates pain perception. A recent study[22] which investigated the effect of female sex hormone on pain perception in healthy, normally menstruating female during the three phases of the menstrual cycle: early follicular, ovulatory, and mid-luteal, demonstrated that the conditioned pain modulation effect of sex hormones varies across the menstrual cycle.

Further, various psychological factors such as depression, anxiety, poor body image, and low self-esteem, which are associated with increased pain during adolescence, have significant and substantial influence primarily on post-pubertal girls.[23] A population based study investigated the depression prevalence and factors influencing the depression in adolescence (11-18 years age) and reported higher prevalence of depression in girls than in boys and a greater influence of pubertal onset on the severity of depressive symptoms in girls than in boys.[24] Further, the study's findings revealed that poor body image and low self-esteem are crucial components in the development of depression and that the intensity of these risk factors is strongest for post-pubertal girls.[24] Since these emotional and psychological factors are characterised by subjective daily variation, this could also have resulted in the greater day-to-day variation in pain perception observed in the 15-18 year female group.

Another possible reason for the large daily fluctuation in pain perception observed in the 15-18 year female group might be due to the higher analgesic consumption reported by these subjects. Since there was no set protocol for the dose, frequency or timing of analgesic consumption, longitudinal within-subject variation in these factors might be driving the large day-to-day variation in pain intensity level seen in this group.

Lastly, the significant residual individual-to-individual differences in WS variability show that there remains an unexplained component to WS pain variability even after adjusting for age and sex. Thus, there remain un-modelled factors which are producing substantial individual differences in day-to-day pain variation. This finding supports the previous study which concluded there are multiple factors which can influence orthodontic pain perception in adolescents besides the age and sex of individual.[4]

Clinical implications

Efficient pain management strategy requires knowledge of not only the mean average pain score across subjects, but also an understanding of how pain varies between and within

each subject. It is generally believed that healthcare professionals lack a common understanding of the meanings behind the scores that pain assessment tools generate, especially in acute care settings.[25]

In our study, results show that there are statistically significant and clinically meaningful individual differences in both the average profile of pain perception and in day-to-day fluctuations around individuals' own trends. Therefore, the common practice of evaluating orthodontic pain by using a single measure obtained on each day can prove misleading; and clinicians managing orthodontic pain should identify such differences as they emerge, and treat patients accordingly.

Limitations and future directions

Our study had several limitations. First, we were not able to control for analgesic consumption in our models. There was no set protocol regarding the dose, frequency and timing of analgesic consumption, and therefore including this factor would very likely have provided misleading information regarding the true relationship between pain and analgesic consumption due to confounding bias. The potential for bias could have been further exaggerated because of likely reciprocal causation between pain perception and analgesic consumption. In future studies, the most appropriate way to study the effect of analgesic consumption on pain would be to experimentally manipulate the amount of analgesic consumption by conducting a randomised control trial. Where only observational data is available there may be some utility to undertaking a simultaneous equation mixed-effects modelling approach wherein both pain perception and analgesic consumption are analysed as joint outcomes. In this approach, correlated random effects (or alternatively a single shared random effect) are introduced across the two outcome equations, thereby acting as a vehicle to capture the likely positive association between the unobserved determinants of each outcome.[26]

Typically this approach will be more convincing when so-called "instrumental variables" known to be predictive of one outcome, but not the other are included in the model.

Second, we did not include the timing of the menstrual cycle in our study. Evidence shows that to examine the influence of female sex hormone on pain perception, a correct determination of menstrual cycle phase (follicular, ovulatory, and mid-luteal) should be based on the serum sex hormone levels analysis and not simple recording of timing of menstrual cycle.[22] Thus, future studies in this direction might better assess the status of menstrual cycle on pain perception based on the analysis of serum sex hormone level rather than just timing of the menstrual cycle.

A third limitation pertains to the fact that only random intercepts and not random slopes were included in the equation for the mean response. While subjects' quadratic time trends are allowed to vary as a function of age and sex and to additionally vary in their overall average levels from subject-to-subject, this may not be sufficient to fully capture the different ways subjects' levels of pain perception evolve over time. Including a random-slope on time would allow for differential rates of recovery across subjects within their age-sex groups and this would seem desirable to at least explore in the current application.[16] However, this modelling extension is not currently implemented in MIXREGLS. Perhaps future studies in this direction would be able to include random time trends as the developers of MIXREGLS program are currently working on upgrading their program (personal communication with the Donald Hedeker) to include random slopes. Alternatively those familiar with the Bayesian estimation framework may choose to fit this extended mixed-effects location scale model using the Stat-JR software[27] as this has been shown to be possible in a recent application of this model to cross-sectional clustered data.[28]

Lastly, our findings are based on pain assessment after orthodontic separators placement. We are not aware of any literature applying similar analyses to studies involving

comprehensive fixed orthodontic appliance. However, such comparisons would be of interest and therefore future studies should also consider fixed orthodontic treatment with comprehensive bands and brackets on all teeth.

Conclusions

Orthodontic pain is a dynamic process with marked individual differences in pain perception, both in terms of individuals' overall average levels of pain, but importantly also in their daily fluctuations in pain perception. Our results shows that females experience greater orthodontic pain as compared to males and that this difference increases with age. 15-18 year female group experienced the greatest mean average pain perception as well as the highest daily fluctuations in pain perception; whereas the 12-15 year females showed the greatest between-subject variations in overall average pain perception.

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We would like to thank Professor Donald Hedeker, Department of Public Health Sciences, University of Chicago, USA for providing valuable support and guidance during the data analysis using his MIXREGLS program. All errors and omissions remain our own.

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Highlights

- We examined pain perception among adolescents after orthodontic separator placement
- A novel “mixed-effects location scale model” was used to analyse pain trajectories
- Mean score and subject variability in pain explored as a function of age and sex
- Subject variability included both between- and within-subject variation in pain
- Age and sex has significant effect on mean score and subject variability in pain

Table 1. Demographic and Clinical Characteristics for each group (n=115)

	12-15 yrs Male group				12-15 yrs Female group				15-18 yrs Male group				15-18 yrs Female group			
	Number (%)				Number (%)				Number (%)				Number (%)			
	28 (24.35%)				29 (25.22%)				28 (24.35%)				30 (26.09%)			
	Age (years)				Age (years)				Age (years)				Age (years)			
	13.5 (SD=1.01)				13.2 (SD=1.04)				16.5 (SD=1.02)				16.6 (SD=0.97)			
	Pain and analgesic data				Pain and analgesic data				Pain and analgesic data				Pain and analgesic data			
Time	VAS score		Analgesic*		VAS score		Analgesic*		VAS score		Analgesic*		VAS score		Analgesic*	
	Mean	SD	Count	%	Mean	SD	Count	%	Mean	SD	Count	%	Mean	SD	Count	%
1 hr	12.5	4.0	0	0%	14	9	6	20.69%	8.6	6.2	4	14.29%	11.1	5.9	4	13.33%
2 hr	20.3	7.0	0	0%	27	13	5	17.24%	25.7	11.6	4	14.29%	26.4	14.5	9	30%
4 hr	27.6	9.4	3	10.71%	36	19	12	41.38%	36.2	12.3	11	39.29%	42.8	19.4	18	60%
12 hr	31.5	12.8	8	28.57%	44	14	15	51.72%	40.9	11.4	15	53.57%	51.1	12.1	25	83.33%
24 hr	37.6	5.2	7	25%	45	16	22	75.86%	45.2	8.9	23	82.14%	53.3	18.0	30	100%
36 hr	36.5	6.4	6	21.43%	43	21	24	82.76%	41.0	15.3	17	60.71%	56.3	23.5	25	83.33%
48 hr	34.5	4.8	3	10.71%	45	16	22	75.86%	44.7	8.6	22	78.57%	52.8	18.3	30	100%
72 hr	26.4	11.2	5	17.86%	43	14	15	51.72%	41.2	12.5	15	53.57%	52.5	13.4	25	83.33%
96 hr	23.5	8.9	1	3.57%	36	19	12	41.38%	34.8	11.9	10	35.71%	42.8	19.4	18	60%
120 hr	17.2	6.3	0	0%	25	13	3	10.34%	24.5	12.0	3	10.71%	29.6	16.4	14	46.67%
144 hr	10.9	4.7	0	0%	11	9	6	20.69%	8.6	7.3	4	14.29%	14.9	8.8	4	13.33%

* Analgesic count shows the number of individuals in each group who consumed analgesic at each time point

Table 2 Results from the mixed-effects location scale model analysis.*

Parameter	Variables	Estimate	Standard Error	z	p	95% Confidence Intervals	
						lower	upper
Mean (Average)	Constant	13.52	0.98	13.75	0.000	11.59	15.45
	Age	-0.61	1.74	-0.35	0.728	-4.02	2.81
	Sex	1.80	1.98	0.91	0.363	-2.08	5.69
	Age-Sex	-0.02	2.74	-0.01	0.994	-5.40	5.36
	Time	9.16	0.26	35.00	0.000	8.65	9.67
	Age*time	3.88	0.39	9.91	0.000	3.11	4.64
	Sex*time	4.32	0.46	9.47	0.000	3.42	5.21
	Age-Sex*time	0.36	0.18	2.03	0.042	0.01	0.71
	Time*time	-0.95	0.03	-37.36	0.000	-1.00	-0.90
	Age*time*time	-0.37	0.04	-9.69	0.000	-0.44	-0.29
	Sex*time*time	-0.42	0.04	-9.63	0.000	-0.51	-0.34
Between-subject (BS) variance	Constant	3.05	0.31	9.89	0.000	2.45	3.66
	Age	0.79	0.42	1.88	0.060	-0.03	1.61
	Sex	1.53	0.40	3.84	0.000	0.75	2.30
	Age-Sex	-0.93	0.55	-1.67	0.094	-2.01	0.16
Within-subject (WS) variance	Constant	2.39	0.20	12.13	0.000	2.01	2.78
	Age	0.22	0.21	1.06	0.289	-0.19	0.63
	Sex	0.69	0.19	3.57	0.000	0.31	1.07
	Age-Sex	0.30	0.27	1.10	0.270	-0.24	0.84
	Time	0.55	0.07	7.34	0.000	0.40	0.70
	Time*time	-0.06	0.01	-7.64	0.000	-0.07	-0.04
Association	Linear association	0.25	0.08	3.24	0.001	0.10	0.39
Scale	Sigma	0.56	0.07	8.51	0.000	0.430	0.688

* Variable coding: Age (0=12-15 years; 1=15-18 years), Sex (0=Male; 1=Female). The Constant (Intercept) repersents Time '0' corresponding to the first wave of data i.e. 1 hour after orthodontic separator placement. The BS and WS variance estimates are on the log scale. The log-likelihood statistic for this model was -4160.6172.

Figure 1 Individual pain trajectories for each group

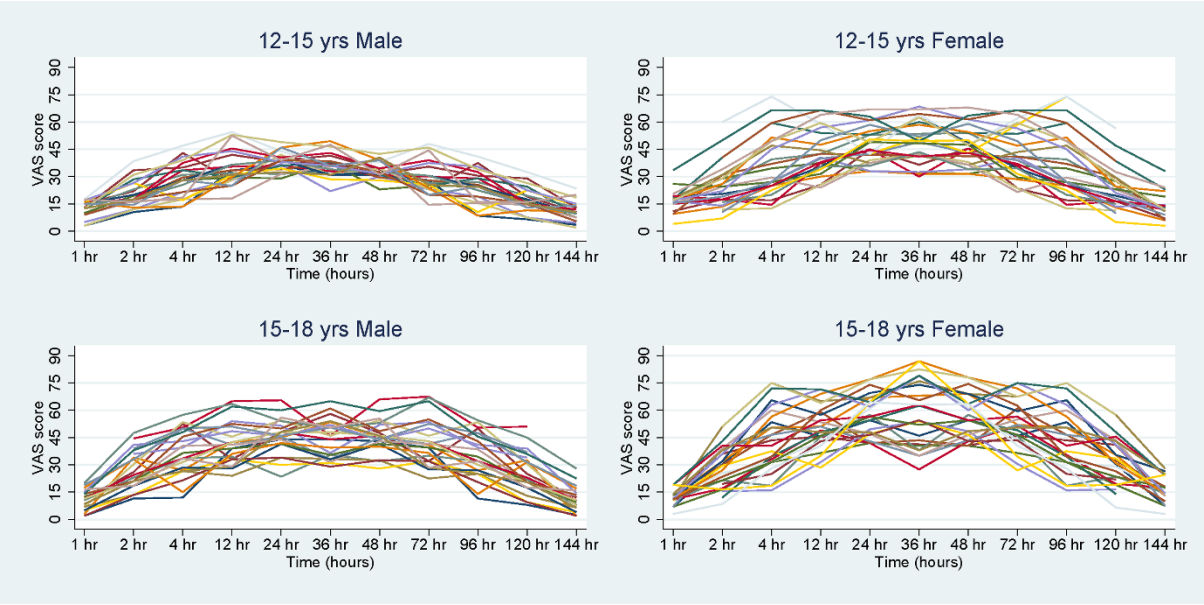
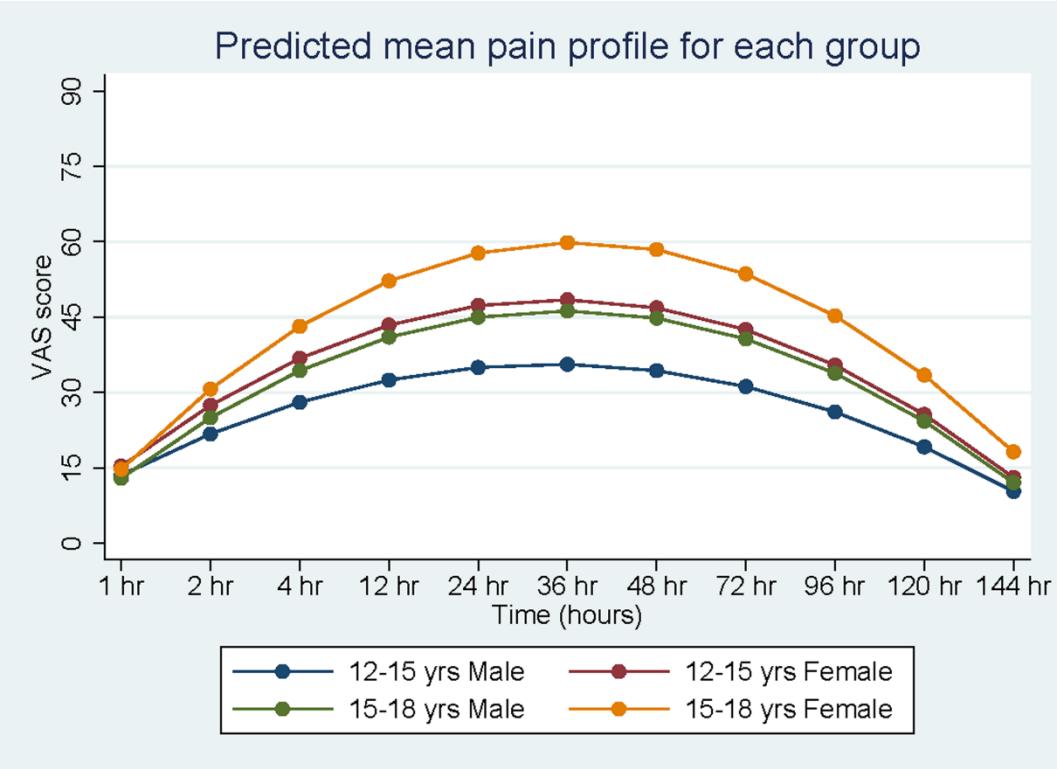


Figure 2 Predicted mean pain trajectories for each group



Online supplementary materials

A. Methodology

Section A1 briefly reviews the standard two-level random-intercept mixed-effects model for continuous response repeated measures data. Section A2 then reviews the mixed-effects location scale version of this model. Section A3 presents the specific mixed-effects location scale model used in the current study. Section A4 describes the model fit and run-in for the current study. Sections A1 and A2 are adapted from Leckie (2014) which itself is adapted from Hedeker and Nordgren (2013).

A1. Mixed-effects model

Let y_{ij} denote the continuous response measurement for subject i ($i = 1,2,\ldots,N$) at occasion j ($j = 1,2,\ldots,n_i$). The standard two-level random-intercept mixed-effects model can then be written as

$$y_{ij} = \mathbf{x}_{ij}^{\mathrm{T}}\boldsymbol{\beta} + \upsilon_i + \epsilon_{ij}, \quad (1)$$

$$\upsilon_i \sim N(0, \sigma_\upsilon^2), \quad (2)$$

$$\epsilon_i \sim N(0, \sigma_\epsilon^2), \quad (3)$$

where $\mathbf{x}_{ij}$ is a vector of covariates, $\boldsymbol{\beta}$ is the associated vector of coefficients, υ_i is the random-intercept effect, and ϵ_{ij} is the residual. The covariates may be time varying or time invariant. The random-intercept effect and residual are assumed normally distributed with zero means and constant variances. The homogeneous between-subject (BS) variance σ_υ^2 measures the variability in subjects' mean responses, having adjusted for the covariates. The

homogeneous within-subject (WS) variance σ_ϵ^2 measures the variability in subjects' measurements about their adjusted mean responses.

The mixed-effects model can be fitted in Stata using the xtreg command with the mle option, or by using the mixed command (Rabe-Hesketh and Skrondal, 2012).

A2. Mixed-effects location scale model

The mixed-effects location scale model proposed by Hedeker and Nordgren, (2013) may be viewed as an extended reparameterized version of the above model and can be written as

$$y_{ij} = \mathbf{x}_{ij}^{\mathrm{T}}\boldsymbol{\beta} + \sigma_{v_{ij}}\theta_{1i} + \epsilon_{ij}, \tag{4}$$

$$\log\left(\sigma_{v_{ij}}^2\right) = \mathbf{u}_{ij}^{\mathrm{T}}\boldsymbol{\alpha}, \tag{5}$$

$$\log\left(\sigma_{\epsilon_{ij}}^2\right) = \mathbf{w}_{ij}^{\mathrm{T}}\boldsymbol{\tau} + \tau_l\theta_{1i} + \tau_q\theta_{1i}^2 + \sigma_\omega\theta_{2i}, \tag{6}$$

$$\theta_{1i} \sim N(0,1), \tag{7}$$

$$\theta_{2i} \sim N(0,1), \tag{8}$$

$$\epsilon_i \sim N\left(0, \sigma_{\epsilon_{ij}}^2\right), \tag{9}$$

where we refer to Equation 4, Equation 5 and Equation 6 as the mean function, the BS variance function, and the WS variance function, respectively.

The mean function (Equation 4) is the same as that in the standard model (Equation 1), except that the random-intercept effect, now referred to as the random-location effect, is parameterized in standardized form, $\sigma_{v_{ij}}\theta_{1i}$. The first term $\sigma_{v_{ij}}$ denotes the square root of the BS variance, while θ_{1i} denotes the standardized random-location effect, $\theta_{1i} \sim N(0,1)$. Note that $\sigma_{v_{ij}}$ is subscripted by i and j to indicate that its value may change across subjects and

occasions. The influence of θ_{1i} on y_{ij} may therefore be amplified or dampened by the magnitude of $\sigma_{v_{ij}}$.

The BS variance function (Equation 5) models the BS variance $\sigma^2_{v_{ij}}$ as a log-linear function of a second vector of subject- or occasion-level covariates $\mathbf{u}_{ij}$ where $\boldsymbol{\alpha}$ denotes the associated vector of coefficients.

The WS variance function (Equation 6) models the WS variance $\sigma^2_{\epsilon_{ij}}$ as a log-linear function of a third vector of subject- or occasion-level covariates $\mathbf{w}_{ij}$ where $\boldsymbol{\tau}$ is the associated vector of coefficients. A quadratic subject-level association is allowed between the unexplained location and scale variability by entering θ_{1i} and its square θ^2_{1i} into the WS variance function as latent covariates with regression coefficients τ_l and τ_q to be estimated. This additional flexibility is useful when the response exhibits floor or ceiling effects, as we then expect a concave relationship between subjects' variances and means whereby subjects with very low or very high means have near-zero WS variances, while subjects with means closer to the middle of the response scale have higher WS variances. A quadratic association is better able to capture such concavity. Finally, a new random effect, denoted θ_{2i} and referred to as the standardized random-scale effect, is included to account for unexplained variation in the WS variance above and beyond the contribution of the covariates. This random effect is assumed normally distributed with zero mean and constant variance σ^2_{ω}.

When $\mathbf{u}_{ij}$ and $\mathbf{w}_{ij}$ each include only a constant and when $\tau_l = \tau_1 = \sigma_\omega = 0$, the above mixed- effects location scale model simplifies to a reparameterized version of the standard two-level random-intercept mixed-effects model with homogeneous variances presented in Section A1.

The use of log-link functions ensures positive variances. However, it makes parameter interpretation less straightforward. In particular, the covariates have multiplicative rather than

additive effects on the variances. In the examples we plot the predicted variance functions to aid their substantive interpretation. This proves especially helpful in interpreting quadratic random-location effects on the WS variance.

As in standard mixed-effects models, the random effects normality assumptions may not necessarily hold and it is prudent to check their plausibility, for example, by inspecting quantile-quantile (Q-Q; normal scores plots) or other graphical plots post-estimation.

The mixed-effects location scale model can be fitted in the **MIXREGLS** software (Hedeker and Nordgren, 2013) which we choose to call from within Stata using the runmixregls command (Leckie, 2014).

A3. Orthodontic pain

In the current paper, we consider the following model for pain

$$y_{ij} = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \beta_3 x_{1i} x_{2i} + \beta_4 t_{ij} + \beta_5 x_{1i} t_{ij} + \beta_6 x_{2i} t_{ij} + \beta_7 x_{1i} x_{2i} t_{ij} + \beta_8 t_{ij}^2 + \beta_9 x_{1i} t_{ij}^2 + \beta_{10} x_{2i} t_{ij}^2 + \sigma_{v_{ij}} \theta_{1i} + \epsilon_{ij}, \quad (10)$$

$$\log\left(\sigma_{v_{ij}}^2\right) = \alpha_0 + \alpha_1 x_{1i} + \alpha_2 x_{2i} + \alpha_3 x_{1i} x_{2i}, \quad (11)$$

$$\log\left(\sigma_{\epsilon_{ij}}^2\right) = \tau_0 + \tau_1 x_{1i} + \tau_2 x_{2i} + \tau_3 x_{1i} x_{2i} + \tau_4 t_{ij} + \tau_5 t_{ij}^2 + \tau_l \theta_{1i} + \sigma_\omega \theta_{2i}, \quad (12)$$

$$\theta_{1i} \sim N(0,1), \quad (13)$$

$$\theta_{2i} \sim N(0,1), \quad (14)$$

$$\epsilon_i \sim N\left(0, \sigma_{\epsilon_{ij}}^2\right), \quad (15)$$

where in the mean function, the pain VAS score y_{ij} is modeled in terms of a quadratic function of time t_{ij} (in days where 0 = bassline), age x_{1i} (0 = 12-15 year-olds; 1 = 15-18 year-olds), and sex x_{2i} (0 = male; 1 = female) and their interactions to allow young, old,

male, and female subjects to differ in baseline pain and to recover at different rates. The random-location effect $\sigma_{v_{ij}}\theta_{1i}$ allows for subject intercept heterogeneity above and beyond that explained by the covariates. The log of the BS variance $\sigma^2_{v_{ij}}$ is modeled as a function of age and sex and their interaction to allow the four groups subject groups to be differentially variable in terms of their mean pain scores, having adjusted for the mean differences between these groups. The log of the WS variance $\sigma^2_{\epsilon_{ij}}$ is modeled as a function of the same two covariates and their interaction, but also the quadratic function of time, to allow the variability of a subject's pain scores about their individual trajectories to differ across the four groups and to change over time. The location effect is entered into the WS variance function to allow for a linear subject-level association between the log of the WS variance and the random-location effect. The random-scale effect $\sigma_\omega\theta_{2i}$ allows for any remaining unexplained variation in WS response heterogeneity across subjects.

The time of peak pain intensity level t_i^{p} is given by differentiating (10) w.r.t. time, setting the resulting equation to zero, and then rearranging. The resulting expression is given by

$$t_i^{\mathrm{p}} = -\frac{1}{2} \times \frac{\beta_4+\beta_5 x_{1i}+\beta_6 x_{2i}+\beta_7 x_{1i}x_{2i}}{\beta_8+\beta_9 x_{1i}+\beta_{10} x_{2i}}, \tag{16}$$

and is a function of age and sex.

A4. Model fit/run-in

A progressive model building strategy was adopted using likelihood ratio tests to guide choice of the final model. Decisions to include/exclude variables in non-nested model comparisons were guided by the Akaike information criterion (AIC) and Bayesian information

criterion (BIC) wherein models with smaller AIC and BIC values were preferred. To further ascertain the fit of the final model, the fitted and observed mean VAS score trajectories were plotted. The plausibility of the random effects normality assumptions were checked via inspection of histograms and quantile-quantile (Q-Q; normal scores plots) plots of their empirical Bayes predicted values.

The mixed-effects location scale model employs full likelihood estimation and so provides valid inference in the presence of missing responses under the missing at random (MAR) assumption.[11] Therefore, all participants who returned the pain questionnaire on one or more occasions were included in the analysis.

Time was coded with the intercept representing the initial status. Age was entered into the model as a dichotomized variable (12-15 year-olds coded '0'; 15-18 year-olds coded '1'). Sex was coded '0' for males and '1' for females. The model fitted to the VAS scores included an intercept, time effects (linear and quadratic), covariates effects (sex, age and age-sex interaction) and covariate-by-time (linear and quadratic) interaction effects to examine whether the four age-sex groups differed in terms of their initial pain severity and rate of change across time (linear time effect) and acceleration/declaration (quadratic time effect). Covariates were also specified for the BS variance and the WS variance sub-models to examine whether the between- and within subjects variability in pain were themselves functions of time and patient characteristics.

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B. Supplementary figures

Figure B1 Model fit evaluation plot for each group

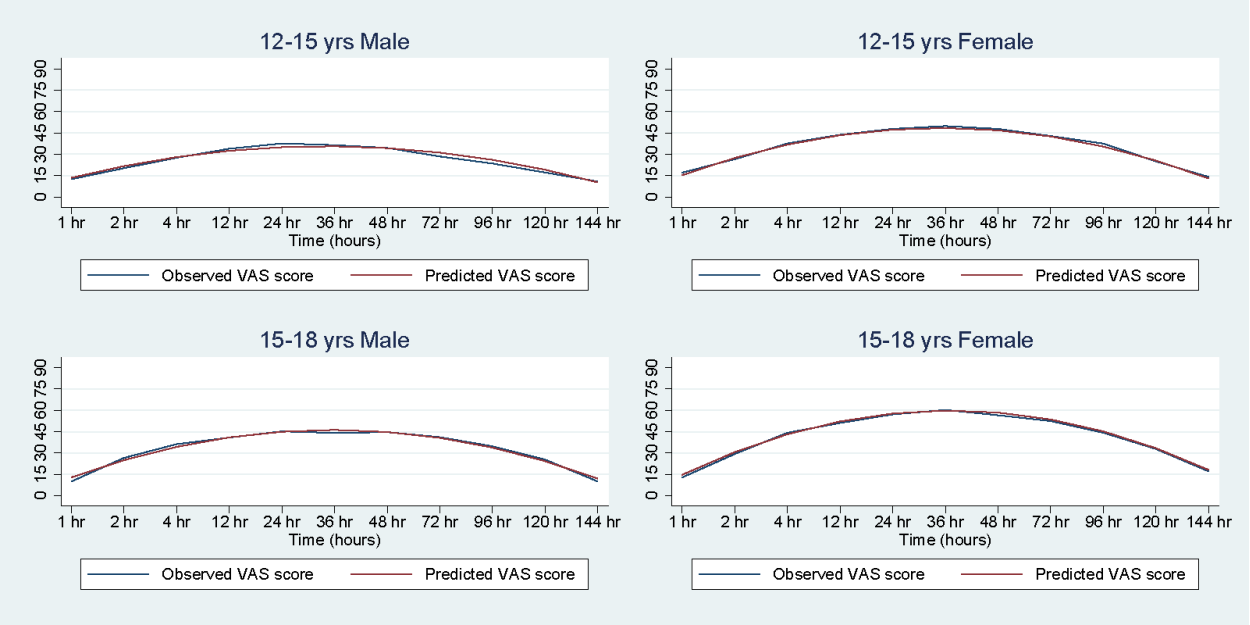
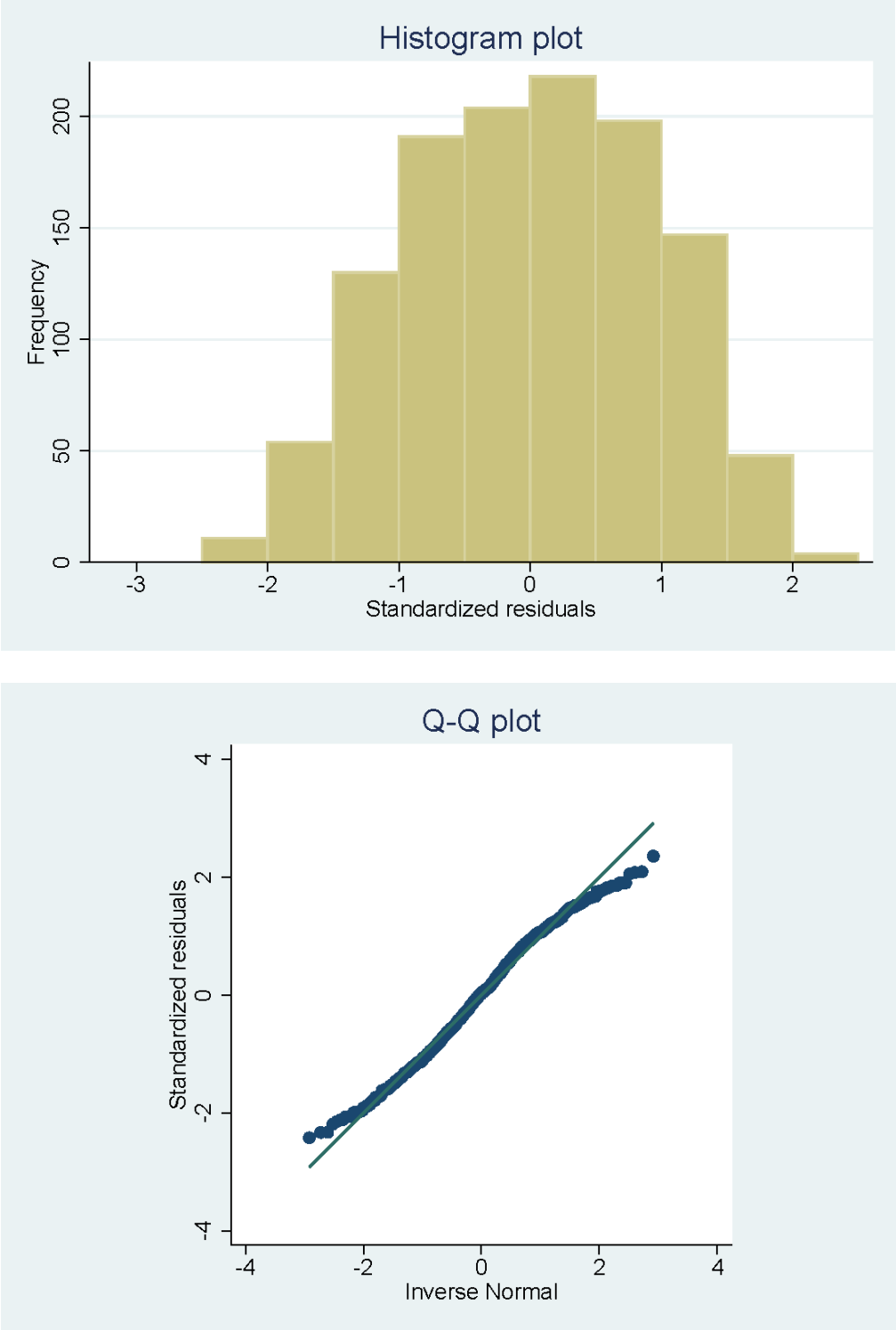


Figure B2 Histogram and Quantile-Quantile (Q-Q) plots for checking the normality assumption of residuals.



C. Supplementary Tables

Table C1 Results showing the pairwise compariosns for the linear trend.

Comparison	Estimate	Standard Error	z	p	95% Confidence Intervals	
					lower	upper
12-15 years Female - 12-15 years Male	4.32	0.46	9.47	0.000	3.42	5.21
15-18 years Male - 12-15 years Male	3.88	0.39	9.91	0.000	3.11	4.64
15-18 years Male - 12-15 years Female	-0.44	0.60	-0.73	0.467	-1.63	0.75
15-18 years Female - 12-15 years Female	4.24	0.41	10.34	0.000	3.43	5.04
15-18 years Female - 15-18 years Male	4.68	0.46	10.13	0.000	3.77	5.58
15-18 years Female - 12-15 years Male	8.55	0.59	14.57	0.000	7.40	9.70

Table C2 Time taken to reach at peak pain intesnity.*

Group	Estimate	Standard Error	z	p	95% Confidence Intervals	
					lower	upper
12-15 years Male	4.83	0.03	145.46	0.000	4.77	4.90
12-15 years Female	4.92	0.03	144.01	0.000	4.85	4.98
15-18 years Male	4.96	0.03	179.46	0.000	4.91	5.01
15-18 years Female	5.10	0.03	152.32	0.000	5.03	5.17

* Time ‘4’ (24 hours), Time ‘5’ (36 hours), Time ‘6’ (48 hours)

Table C3 Results showing the pairwise compariosns for time taken to reach at peak pain intesnity.*

Comparison	Estimate	Standard Error	z	p	95% Confidence Intervals	
					lower	upper
12-15 years Female - 12-15 years Male	-0.09	0.05	-1.82	0.068	-0.18	0.01
15-18 years Male - 12-15 years Male	-0.13	0.04	-3.00	0.003	-0.21	-0.04
15-18 years Male - 12-15 years Female	0.04	0.04	0.97	0.333	-0.04	0.13
15-18 years Female - 12-15 years Female	0.18	0.05	3.82	0.000	0.09	0.28
15-18 years Female - 15-18 years Male	0.14	0.04	3.24	0.001	0.06	0.23
15-18 years Female - 12-15 years Male	0.27	0.05	5.73	0.000	0.18	0.36

* Time ‘4’ (24 hours), Time ‘5’ (36 hours), Time ‘6’ (48 hours)