Summary The purpose of this study was to use salivary cortisol levels, pressure pain threshold (PPT) and Spielberger’s State-Trait Anxiety Inventory for Children (STAIC) to assess stress, anxiety and pain during the expansion and retention phase of rapid maxillary expansion (RME) in children and investigate whether these parameters are associated with gender or skeletal maturity stages. STAIC was used to assess the anxiety levels of the children. Salivary samples were collected for stress hormone determination. Visual Analog Scale was used for pain determination. Pressure pain threshold (PPT) was measured by using algometer. Data collection was performed a week before RME treatment (T0), at the day of the expansion appliance was bonded (T1), at the days of 1st, 4th, 7th, 14th, 25th, 36th activations of expansion screw (T2, T3, T4, T5, T6, T7) and after the retention period of 3 months (T8). The results of this study showed that the differences were statistically significant within-day ($P < 0.001$) and within-hours ($P < 0.001$) in cortisol levels during treatment. PPT levels were statistically significant within sex differences and skeletal maturity stages ($P < 0.05$). State-trait anxiety scale scores were similar with respect to gender ($P > 0.05$). There were statistically significant differences of state-trait anxiety levels between pre and post-treatment stages ($P < 0.05$). The maximum number of patients reporting pain were days at T3 and T4. From day T5 the percentage of patients reporting pain then gradually reduced. Based on the findings of this study, it has been shown that RME leads to changes in patients’ state-trait anxiety and cortisol levels.