Steroids Analysis of Human Saliva: Testosterone a New Way to Identify Gender

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Abstract

Identification of gender in a forensic case can significantly narrow down the identity of individuals involved. Steroid hormones are a group of naturally occurring compounds that vary in concentration depending on gender. Because of the blood stream connection to saliva production, steroids from the blood circulation enter saliva. We hypothesize that steroid hormones in saliva can be used to determine gender. To investigate steroid hormones levels in saliva, we used Ultra Performance Liquid Chromatography with Electrospray Ionization Triple-Quadrupole Mass Spectrometer (UPLC-ESI-TQ-MS). We collected six human saliva samples from females and used UPLC-ESI-TQ-MS to look for four steroid hormones: progesterone, testosterone, estrone, and estradiol. Testosterone was detected in males, but was not detected in females. However, progesterone, estrone, and estradiol are found in both males and females. These results demonstrate that testosterone can be used to identify gender. In summary, our preliminary findings suggest testosterone could be a potential candidate in forensic science to help identify the gender of an individual without the use of DNA.

Methods

Saliva samples were collected from six different human subjects: 3 men and 3 women. The saliva samples where labeled with numbers to blind the gender of the subjects. Saliva was next concentrated, filtered, and run on a UPLC-ESI-TQ-MS. The samples where then analyzed for four steroid hormones: Progesterone, Testosterone, Estrone, & Estradiol. After steroid analysis was completed, samples were un-blinded.

Ultra performance liquid chromatography (UPLC) uses a column and solvents to separate and identify chemicals based on their polarity. The time it takes a given chemical to pass through the column is a known retention time (RT).

UPLC has 2 phases that control a chemical by its polarity:
- Stationary phase: Column that is packed with nonpolar beads
- Mobile phase: Polar liquid (solvent) that moves through the Miles

Chromatography with Electron Spray Ionization Triple-Quadrupole Mass Spectrometer with a scan of 3.200 amu. The ion source was set to a positive ion mode.

Results

In this study we used UPLC-ESI-TQ-MS to determine the presence of four different steroid hormones. Our chromatography results showed that progesterone is present in samples W1, W2, and M1, but it was not present in W3, M2, or M3. Testosterone, a down stream metabolite of progesterone, was detected in W1, W2, and M1, however it was not detected in W1, W2, or W3. Estrone, a type of estrogen, was present in W1 and M2 but not in W1, W2, or W3. Estradiol, a metabolite of testosterone, was detected in W2, M1, and M2 but not in W1, W2, or W3. These results indicate that in saliva testosterone can be used to identify a humans gender.

Conclusions

- Our research suggests that testosterone can be used to identify gender in saliva.
- The first report of gender identification through saliva in humans.
- Testosterone could be used as an alternative to DNA gender identification, which would be beneficial in forensic science.
- Preliminary test to provide further characteristic of the evidence.
- Potentially faster method to determine gender.
- Progesterone, estrone, and estradiol were found in males and females, therefore they can be used to determine gender through saliva.

What’s next?

- Further studies with larger sample sizes are needed to confirm these findings.
- Identify gender using saliva left on food and drinks.

Citations

2. Babic, D. and J. R. Wilkinson. A review of saliva: Normal composition, function and applications. My goal is to become a scientist and this project helped introduce me to this career.

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My Experience:

- Changed this project because it combines science with real life applications. My goal is to become a scientist and this project helped introduce me to this career.
- The hardest aspect about the project was learning about the UPLC-ESI-TQ-MS, due to the fact that it is a complex machine. However, my mentor worked with me and now I have a basic understanding of the machine.
- My favorite part about this project was that I participated in the entire experiment. I was able to create this poster with minimal help from my mentor. Collectively, this was a great learning experience.
- What I learned in this project will be beneficial for me in college because it is an area that I am interested in.

Figure 1. Salivary glands. Saliva is a biological fluid produced by salivary glands. Saliva is 99% water, but also contains steroid hormones, and other compounds. It is produced from 3 types of salivary glands: the parotid (P), submaxillary (S), and sublingual (L) glands.

Figure 2. UPLC-ESI-TQ-MS chromatograms of the 6 saliva samples. Progesterone, testosterone, and estradiol were detected in both males and female saliva. Testosterone was only present in male saliva, revealing that it can be used to identify gender.

Figure 3. Simplified steroid metabolism in humans. Progesterone, testosterone, estrone, & estradiol are highlighted in green to indicate targeted steroids analyzed in this study.

Figure 4. My Experience: I chose this project because it combines science with real life applications. My goal is to become a scientist and this project helped introduce me to this career.