Preliminary Data at Huanchaquito Las Llamas: Elucidating Environmental Instability and Cortisol Production leading up to Ritual Execution

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INTRODUCTION

Excavations at the Peruvian northern coastal site of Huanchaquito-Las Llamas (HLL) revealed the largest mass human sacrifice event in the Americas, with more than 300 sacrificed Muchik women, children, and camelids. Dated to the Chimú's imperial decline (circa 1450 CE), these women and children were drawn from multiple regions and ethnic groups throughout the empire (Figure 1). While the motivation for such a massive sacrifice is a subject for further research, the archaeological evidence suggests that it was associated with massive flooding and various environmental changes over the course of months linked to a mega El Niño climatic event (Prieto et al. 2019

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Archaeological Site

Huanchaquito Las Llamas

METHODS

Initial Preparation

Hair samples from Huanchaquito Las Llamas were cleaned ultrasonically in ddH20 and followed by ethanol and another round of ddH20. Hair samples were then weighed, and scissor minced inside their microtube to reduce static and risk of sample loss from transport.

Cortisol Extraction

- 1ml of HPLC-Grade methanol was added to the minced samples
- Samples were incubated for 18-24 hours at room temperature with constant inversion
- Samples were centrifuged at 10,000 rpm for 5 minutes
- 0.6 ml of the supernatant to a clean microtube
- Samples were then freeze-dried

Slope Analysis

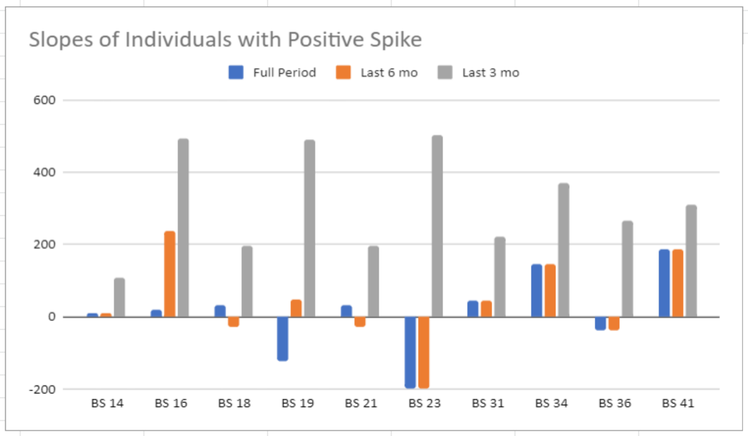
The constant growth of hair allows us to treat the cortisol level like a continuous function over time. First we take the average rate of change for the entire time period for a single individual. If they increased 10 points over five months, their average rate of change is 2 units/month. By focusing our interval, we modeled the changes in cortisol level on a month-to-month basis, which allows us to create another linear representation, known as a first order derivative.

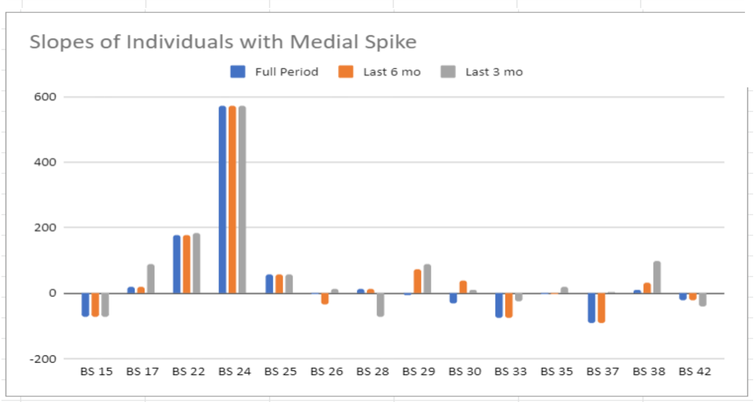
This model is a much more accurate representation of the individual on a smaller scale, as it represents the change as the individual experienced it. If an individual's level spikes in one month and falls in the next, the average over the period will be close to 0, but by focusing in and interpreting the slopes month by month, we can much more easily see the changes that occur. This matters especially for data that experiences varying increases and decreases.

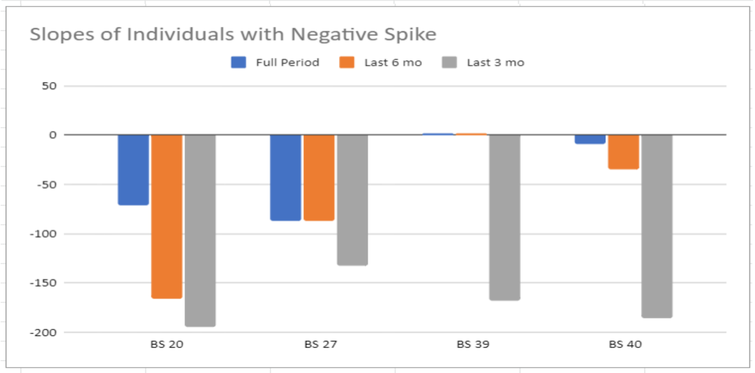
Cortisol Assay Procedure (Meyer et al. 2014)

1. The procedure was conducted using a Salimetrics enzyme-linked immunosorbent assay (ELISA).
2. 24 mL of Assay Diluent was pipetted into the microtube for 1.5 hours at room temperature.
3. Microtitre Plate was brought to room temperature.
4. Enzyme Conjugate 1:1600 was diluted by adding 15 uL to the 24 mL Assay diluent.
5. The plate was then placed on a rotator for 5 minutes at 500 rpm and incubated at room temperature for 1 hour and then subsequently washed 4 times with 1X (100 mL of wash buffer concentrate to 900 mL deionized H20) wash buffer.
6. 200 uL of TMB substrate solution was added to each well.
7. The mix was placed on a plate rotator for 5 minutes at 500 rpm and incubated in a dark room for an additional 25 minutes.
8. Add 50 uL of Stop Solution to the sample and placed on plate rotator for 3 minutes at 500 rpm and read at 450 nm.
9. Samples were then reconstituted at 200 uL and stored at -20 C for future assays

RESULTS

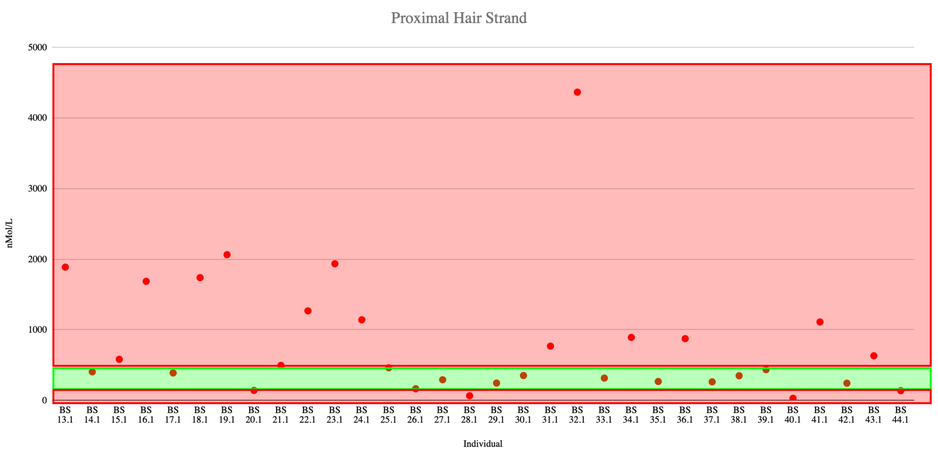






Graph 1: Bar graph depicting positive spikes in cortisol
Graph 2: Bar graph depicting medium, yet still positive spikes in cortisol
Graph 3 Bar graph depicting negative spikes in cortisol

Graph 4: Proximal Hair Strand Cortisol Results. Data points inside the green box denote clinically typical cortisol results. Data points inside the red boxes denote atypical cortisol results



BS #	Age	Sex	Cut Mark on Sternum Y/N	Description
BS 13	6 years		n	0
BS 14	12-15 years		y	one transverse bisecting cut
BS 15	12 years	female	y	one transverse bisecting cut
BS 16	12 years		n	0
BS 17	15 years		y	one transverse bisecting cut
BS 18	10 years		y	one transverse bisecting cut
BS 19	11 years		y	one transverse bisecting cut
BS 20	12 years		y	one transverse bisecting cut
BS 21	11 years		n	0
BS 22	5 years		y	one transverse bisecting cut
BS 23	12 years		y	two transverse cut marks; one bisecting; one partial
BS 24	10 years		y	one transverse bisecting cut
BS 25	15 years		y	two transverse cut marks; one bisecting; one partial
BS 26	6 years	possible male	n	0
BS 27	9-10 years		y	one transverse bisecting cut
BS 28	9-10 years	possible male	n	0
BS 29	15 years		y	one bisecting cut mark across two parallel segments
BS 30	11 years	possible female	y	one transverse bisecting cut
BS 31	10 years		n	0
BS 32	9 years		y	one transverse bisecting cut
BS 33	9 years	possible male	y	one transverse bisecting cut
BS 34	6.5-7 years		n	0
BS 35	7.5-8 years		y	one transverse bisecting cut
BS 36	8-9 years	possible male	y	one transverse bisecting cut
BS 37	9 years		y	one transverse bisecting cut
BS 38	7 years	possible male	y	one transverse bisecting cut
BS 39	12 years		y	one transverse bisecting cut
BS 40	9-10 years	possible female	y	one transverse bisecting cut
BS 41	6-7 years		y	two bisecting cut marks
BS 42	7-8 years		n	0
BS 43	6.5 years	possible male	y	one transverse bisecting cut

Table 1: Demographic Data including estimated age, sex, sacrificial cut, and description.

Proximal Hair Strand Cortisol results suggest that most individuals included in this sample have atypical cortisol production in the final month leading up to ritual execution. Those that demonstrate typical cortisol levels are inside the green box. Based on slope analysis from cortisol, there are more individuals with positive spikes compared to negative drops in cortisol production overtime in the months leading up to sacrifice. While the graphs show three bars depicting the full period of hair, last 6 months, and last 3 months, slope analysis was run for the entire sample of hair per individual. These graphs demonstrate exciting new research avenues.

DISCUSSION

- These figures demonstrate a wide spread of data points, with some individuals far above and far below the typical values. Most individuals showed some kind of skew, with the more prevalent being towards the higher end.
- The standard deviations illustrated generally the same trend, with higher means having higher standard deviations. The conditional chart indicates whether each individual's mean and standard deviation pair fell within typical bounds for cortisol levels. This binary model served as the foundation for Chi-squared tests.
- We also treat the timeline as a variable, since the consistent growth of hair gives a reliable timescale. We used the secant slope of the data (the average over the entire curve) that showcased a weak trend. However, when we took the slope of the final 6 months and the final 3 months, a pattern visibly emerged.
- The highs became higher, and the lows became lower. This "extremification" of the data over time explains the extreme values we were seeing. From there, we took the average rate of change at every interval for every individual in order to create second order derivatives for the curves created. Taking an average of the "accelerations" of the data returns a value of 58 units whereas its parent function demonstrates an increase of 750 points over five months, assuming some degree of consistency.
- This 58 value does not account for the sharp negative drops; calculating an absolute value of the "acceleration" chart brings the mean value to 138 units. This value's parent function sees over 1,000 points of growth over only a four-month period. Of the 32 individuals who had enough data to establish a trend, only 7 had cortisol mean/slope/acceleration groups that fell within typical levels and 25 outside typical levels.

CONCLUSIONS

- Between four and six months antemortem, 20 of the individuals showed a spike or drop in cortisol levels, with values trending upwards or downwards almost exponentially.
- Prieto et al. (2019) noted that the archaeological evidence points towards catastrophic environmental effects from El Niño resulting in the mass human sacrifice event. The data strongly suggests a stress inducing-period beginning around 4-6 months antemortem and sustaining until time of death.
- According to Theall et al. (2016), which focused on child victims and witnesses to household violence, both increases and decreases in cortisol can be indicators of adversity in children. Similarly, the data align with individualized data.
- While more evidence (and is forthcoming!), the preliminary cortisol slope analysis suggests that the consistent spike and drops around the same months suggest episodic stress periods that the community experienced; had the data shown no clear pattern, the data would suggest that there was not a shared episodic stress period experienced by the community. It is important to note that everyone embodies stress differently, so while these data are not conclusive, they strongly suggest that the catastrophic El Niño may have been an attributing factour to their selection.

REFERENCES

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