

Quantitative Variation of Time-Activity Curve Derived Parameters with Regional Variation of Background Region of Interest, in Salivary Scintigraphy: A Retrospective Comparative Evaluation

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Abstract

Aim and Objective: Quantitative analysis of time-activity curve (TAC) derived from ^{99m}Tc-pertechnetate salivary gland scintigraphy, used in the evaluation of salivary gland function, is almost always corrected for background count. The objective of this study was to: (a) Examine the effect of the alteration of anatomical position of the background region of interest (ROI) on the (1) semi-quantitative parameters, i.e., upslope (Us) and stimulated downslope (Ds), derived from TAC and (2) on minimum and maximum pixel counts of pixel histogram of background ROI. (b) Examine the effect of minimum and the maximum pixel counts of the background ROI on the values of Us and Ds.

Materials and Methods: In this retrospective study, 16 patients were included who had undergone ^{99m}Tc-pertechnetate salivary scintigraphy for various reasons but had normal salivary scintigraphy reports. Three different sets of background in different anatomical positions were chosen: (i) A pair of supraclavicular background ROI, (ii) an isolated central rectangular background ROI in brain, and (iii) an isolated suprathyroid ROI. We then calculated two important semi quantitative parameters, i.e., Us and Ds from the TAC of the salivary glands. Individual TAC was generated against each set of the above-mentioned backgrounds. Individual pixel histogram was also generated against each TAC. Minimum and maximum pixel count of the generated background pixel histograms was identified. Comparative studies were made among the afore-mentioned variables.

Results and Conclusion: The values of Us and Ds derived from respective background corrected TAC matched well among each other irrespective of the altered anatomical positions of the background ROI. The minimum pixel counts per second (CPS) of the background pixel histogram varied with the change in anatomical position of the background ROI but it did not affect the values of Us and Ds. In contrast, the maximum pixel CPS of the background pixel histogram varied inversely with the values of Ds, although the value of Ds, showed no variation with the change in position of the background ROI.

Key words: Maximum pixel count per second, Minimum pixel count per second, Partial volume effect, Pixel histogram, Rate constant, Region of interest, Stimulated downslope, Upslope

INTRODUCTION

The aim of the present study was to compare the influence of regional variation of the background region of interests (ROIs) on the uptake and secretion related semi-quantitative

parameters of the salivary glands. We also aimed to assess the correlation among the derived semi-quantitative parameters with minimum and maximum pixel count of background ROIs in different anatomical positions. These two parameters were assessed in during salivary scintigraphy through: (a) accumulation (uptake) of ^{99m}Tc-pertechnetate and (b) stimulated secretion (excretion) of ^{99m}Tc-pertechnetate. The accumulation in the salivary gland is usually assessed as the ratio of gland counts to background counts at the point of maximum activity and the clearance or secretion is defined by subtraction of the ratio of salivary gland counts to background counts at the

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nadir (achieved after lemon juice stimulation), from that of gland to background counts at the point of maximum counts.^[1] Thus, it is evident from the definition that the selection of background counts is of critical importance for calculating both the parameters and that over or under subtraction of the background counts while drawing the ROI would falsely elevate or reduce the clearance or secretion index of the salivary glands.

ROI in the functional imaging is defined as a closed boundary surrounding some features or area, from which some statistics (such as counts) are derived. There are many ways to determine the shape and position of the background ROI. In salivary scintigraphy, to standardize the level of salivary gland accumulation and secretion, the correction of vascular background is recommended. However, the area chosen for the correction of vascular background varies widely, for example, skull, midline brain, the region above and lateral to the thyroid gland, and bilateral supraclavicular region all have been used and recommended from time to time by different authors.

The required semiquantitative parameters, i.e., upslope (Us) and downslope (Ds) were calculated from each time-activity curve (TAC) derived from salivary scintigraphy following subtraction of backgrounds in different anatomical positions. In addition, against each set of TAC, a pixel histogram was produced. The influence of regional variation of the background ROIs on the salivary gland uptake and secretion related semi-quantitative parameters was calculated first. The correlation among the derived semi-quantitative parameters with minimum and maximum pixel count of background ROIs in different anatomical positions was assessed next.

At the further basic level, the counts per pixel in the background ROI are calculated and subtracted from the counts per pixel in the organ of interest. The number of counts per pixel per second is proportional to the radioactivity concentration. ROI data from a series of frames can be used to create a TAC showing radiotracer concentration as a function of time for that ROI. TAC can be generated from each pixel, and it usually shows average changes in the count density within the ROI. Thus, the number of counts in a particular pixel is replaced finally by the semi-quantitative parameters like parameter (Ds, Us, etc.) derived from its TAC.

MATERIALS AND METHODS

We studied retrospectively those patients who had been referred to the nuclear medicine department for the purpose of ^{99m}Tc-pertechnetate salivary scintigraphy due to various reasons. However, normal functioning salivary

glands were detected as the scintigraphy outcome in these patients. Sixteen such patients were included in the study. Among these 16 patients, five patients had been referred due to recurrent swelling of parotid glands, ten patients had symptoms of mild dryness of mouth, and one patient was referred from surgery department due to failure to detect ductal orifice.

Salivary Scintigraphy Protocol

Dynamic salivary gland scintigraphy was performed following the injection of 370 MBq ^{99m}Tc sodium-pertechnetate with the gamma camera and data analysis system dual-head gamma camera (Siemens), equipped with low energy high-resolution parallel-hole collimator was used for imaging. Sequential anterior images of the salivary glands were obtained at 20 s per frame^[1,2] for 50 min. Forty minutes after the injection of the tracer, 2 mL of lemon juice was administered orally for salivary glands stimulation. We selected the frame after 20 min with optimum counts, have drawn ROI on each of the salivary glands (parotid and submandibular). Then for each patient, we applied different sets of background ROIs. Against each set of background ROI, TAC for each of the parotid gland and submandibular glands were generated.

Generation of Background ROI and TACs

The following sets of different background ROIs were generated for the study: (a) A pair of supraclavicular ROIs, and (b) an isolated central rectangular background ROI in brain, and an^[3] isolated suprathyroid ROI (superior and lateral to thyroid gland). System generated simple geometric ROI was drawn to avoid error in the manual ROI. Standard rectangular ROI was drawn for the background and oval-shaped^[2] ROIs for the salivary glands. When number of backgrounds was more than one, the average number of pixel of the background ROIs was considered for calculation.

Count Normalization According to Area and Number of Pixels

Counts in the target salivary gland ROI were normalized^[4] with respect to the pixel count of the background and then counted in the target organ was calculated by the following formula:

$$C=(CR-CB)\times PR/PB$$

CR=COUNTS IN THE TARGET ROI

CB=COUNTS IN THE BACKGROUND

PR=AREA OF TARGET ROI IN PIXELS

PB=AREA OF BACKGROUND ROI IN PIXELS

C=COUNTS IN THE TARGET ROI AFTER BACKGROUND SUBTRACTION correction factor is the ratio of pixel counts of target ROI and the pixel counts of background ROI. When multiple

backgrounds were selected, the correction factor is the ratio of pixel count of the salivary gland ROI to the average pixel count of background (calculated by dividing the sum of pixel numbers of all the backgrounds selected by the number of backgrounds selected).

Generating the TACs

Subsequently, the respective curves of background and salivary organ of interest were generated sequentially using the software, as follows:

- Curve 1: Was drawn from either the TAC of the selected isolated background or the sum of selected background ROIs
- Curve 2: Was generated by averaging of the background TAC when multiple backgrounds are used. This was undertaken by dividing the curve with the constant number, equal to the number of background ROIs
- Curve 3: The provisional TAC of the organ of interest
- Curve 4: Curve 3 is then normalized by multiplying it by the correction factor to generate the curve 4
- Curve 5: Is the final target salivary gland TAC. This is generated by subtracting Curve 2 from curve 4.

Curve 5 was subjected to a linear fit. The knowledge of the physiological activity of a normal salivary gland as well as physiological concentration of radiopharmaceutical with time allows us to fit a mathematical function to the TAC curve. Analysis of curve slope and shape following fit of

the mathematical function helps to extract the different functionally significant parameters as described below:

Calculation, analysis, and comparison of two parameters U_s and D_s of curve 5 were undertaken next.

The activity uptake rate, represented by the U_s , is a simple measure of the efficacy of the gland to extract the radiotracer from the circulation. The TAC of dynamic scan of normal salivary gland is more or less linear during the interval between 0 s and 240 s due to progressive accumulation of tracer. Following the application of the linear fit^[5] in the desired portion of TAC curve one can calculate the slope or direction-coefficient (D_c) at the desired points of time in the curve between a specific time interval. The U_s represents the D_c between 2 and 4 min after injection [Figures 1-3]. The stimulated D_s was defined as the D_c between the application of citric acid and minimum uptake or the lowest value after stimulation [Figures 4, 5 and 6]. The next step was to generate pixel histograms in the same way as the TAC was generated against each set of backgrounds. For each such set of histogram, the negative peaks indicated the background [Figures 7-9]. The range of pixel counts per second (CPS) along X-axis was noted.

Observation

Numerical variables of the whole cohort were subjected to Kolmogorov–Smirnov goodness-of-fit test and found



Figure 1: a1 value represents the upslope value. Background is central brain

Pal and Das: Quantitative Variation of Time-Activity Curve Derived Parameters with Regional Variation of Background Region of Interest, in Salivary Scintigraphy: A Retrospective Comparative Evaluation

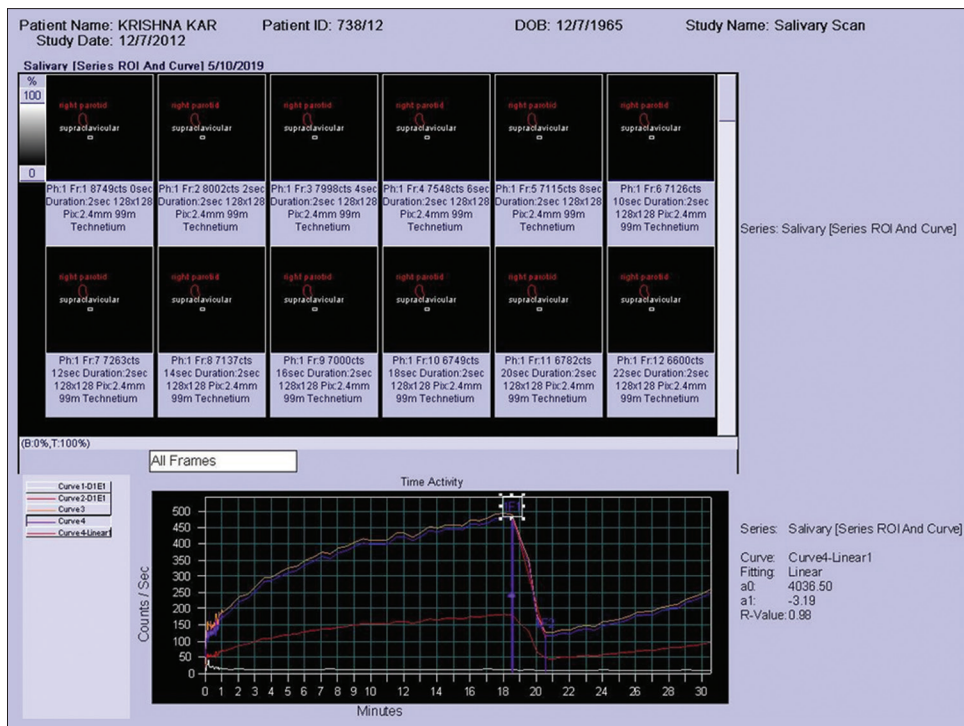


Figure 2: a1 value represents upslope value. Background is suprathyroid



Figure 3: a1 value represents the downslope. Background is central brain

to be normally distributed. Comparison of the four parameters (U_s , D_s , minimum CPS, and maximum CPS) between three background related subgroups by one-way analysis of variance and *post hoc* testing following

ANOVA by Tukey's test [Tables 1 and 2] show that the change in anatomical position of the background had no statistical significant effect on the U_s and D_s derived from TAC.



Figure 4: a1 value represents the upslope value. Background is bilateral supraclavicular



Figure 5: a1 value represents the downslope value. Background is bilateral supraclavicular

Comparison of the Four Parameters between the Three Background Related Subgroups One-way Analysis of Variance

However, it was observed that minimum CPS of the background pixels in pixel histogram varies with the change in the anatomical position of background ROI, but this

change had not affected the semi-quantitative parameters of TAC [Tables 3 and 4]. On the other hand, the maximum CPS of background ROI did not change with the positional change of background, but it affects the Ds inversely in contrast to the Us that remains largely unaffected by the maximum counts [Tables 3 and 4].

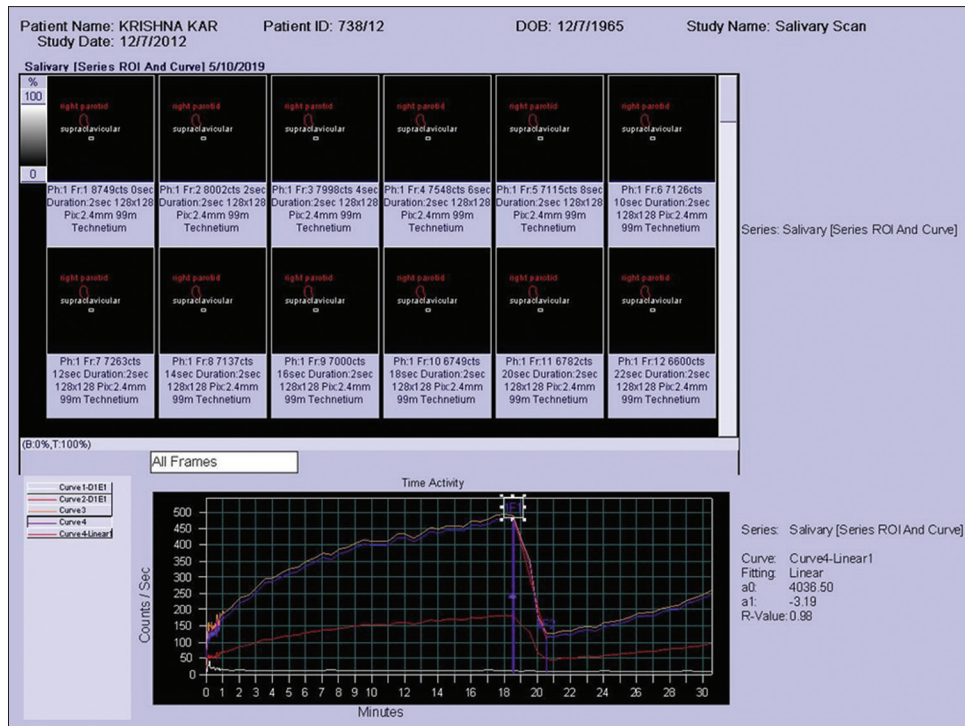


Figure 6: a1 value represents the downslope value. Background is suprathyroid

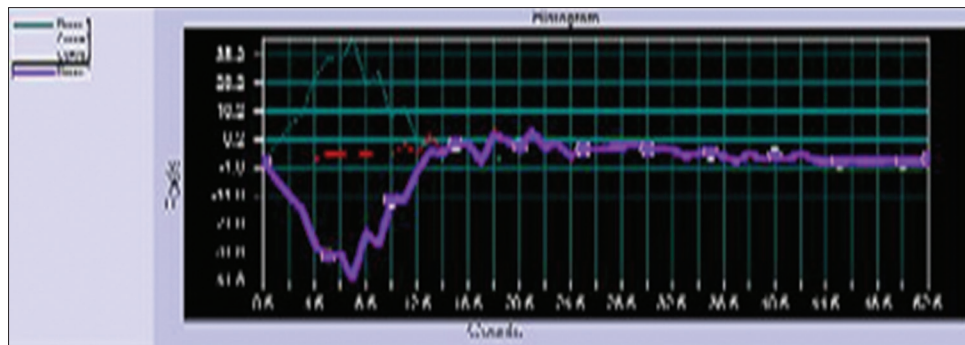


Figure 7: Pixel histogram of bilateral supraclavicular background. Curve below the baseline is of the background

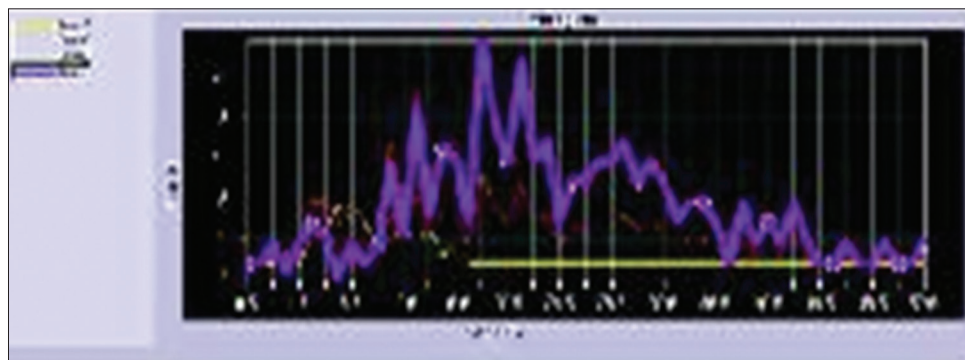


Figure 8: Pixel histogram of suprathyroid background. Curve below the baseline is of the suprathyroid background

DISCUSSION

Thus, as observed in the study, change in the anatomical position of background ROI does not seem to affect the

quantitative parameters of the salivary glands significantly. The two most important factors that determine the radioactivity quantification in an organ are (1) photon interaction and (2) partial volume effect (PVE). Both

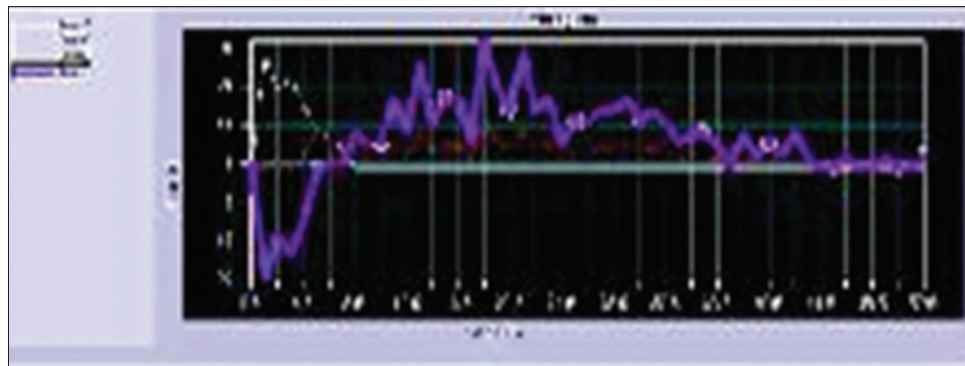


Figure 9: Pixel histogram of central brain background. Curve below the baseline is of central brain background

Table 1: *Post hoc* testing following ANOVA by Tukey’s test

Dependent Variable	(I)	(J)	Mean difference (I-J)	Standard error	Significant	95% Confidence interval	
	SubGr	SubGr				Lower bound	Upper Bound
Us	1	2	-0.00300	0.02952	0.994	-0.0745	0.0685
		3	-0.00975	0.02952	0.942	-0.0813	0.0618
	2	1	0.00300	0.02952	0.994	-0.0685	0.0745
		3	-0.00675	0.02952	0.972	-0.0783	0.0648
	3	1	0.00975	0.02952	0.942	-0.0618	0.0813
		2	0.00675	0.02952	0.972	-0.0648	0.0783
Ds	1	2	-0.0006	0.1907	1.000	-0.463	0.462
		3	-0.0950	0.1907	0.873	-0.557	0.367
	2	1	0.0006	0.1907	1.000	-0.462	0.463
		3	-0.0944	0.1907	0.874	-0.557	0.368
	3	1	0.0950	0.1907	0.873	-0.367	0.557
		2	0.0944	0.1907	0.874	-0.368	0.557
Min CPS	1	2	-4.781	13.662	0.935	-37.89	28.33
		3	-35.031*	13.662	0.036	-68.14	-1.92
	2	1	4.781	13.662	0.935	-28.33	37.89
		3	-30.250	13.662	0.080	-63.36	2.86
	3	1	35.031*	13.662	0.036	1.92	68.14
		2	30.250	13.662	0.080	-2.86	63.36
Max CPS	1	2	-6.654	21.574	0.949	-58.94	45.63
		3	-39.564	21.574	0.170	-91.85	12.72
	2	1	6.654	21.574	0.949	-45.63	58.94
		3	-32.909	21.574	0.289	-85.20	19.38
	3	1	39.564	21.574	0.170	-12.72	91.85
		2	32.909	21.574	0.289	-19.38	85.20

* The mean difference is significant at the 0.05 level. CPS: Counts per second. Numerical variables of the whole cohort were subjected to Kolmogorov–Smirnov goodness-of-fit test and found to be normally distributed

parotid glands and submandibular glands are very superficial structures and so are their background structures. By altering the anatomical positions of background ROIs, the physical factors such as attenuation and scattering do not affect their counts due to less depth of the origin of the radioactivity. Besides, the PVE does not contribute to play a significant role in this case scenario, because the PVE^[6,7] is most important while quantifying the activity within objects of sizes less than twice the full width half maximum spatial resolution of the imaging system. Organs that are smaller or elongated will have greater PVE.^[7] The size of both the salivary glands and background structures is usually more than the full width at half maximum of the system, as described earlier. Furthermore, in the present analysis, it was observed that although minimum CPS of

the background pixels in pixel histogram varied with the change of the anatomical position of background ROI, this change did not affect the semi-quantitative parameters of TAC curve. Furthermore, although CPS determine the uptake, the change in the minimum CPS of the background ROI does not affect the semi-quantitative parameters.^[7]

This observation can be explained in the following manner: Following tracer administration into the central compartment, initially there is very fast decay in the distribution phase and the efflux rate constant k_{12} plays the dominant role.^[8] The minimum CPS of any region solely depends on k_{12} but while the drug is distributing from the central to the peripheral compartment, there is drug returning back to the central compartment at a certain

Table 2: Comparison of the 4 parameters between the 3 background related subgroups One way analysis of variance

Semi-quantitative parameters	SS	df	MS	SS	df	MS	F	P
Us	0.00	2	0.000	0.3	45	0.007	0.057227	0.944
Ds	0.10	2	0.048	13.1	45	0.291	0.164402	0.849
Min CPS	11547.26	2	5773.630	67198.1	45	1493.292	3.866377	0.028
Max CPS	14360.52	2	7180.261	167552.6	45	3723.391	1.928420	0.157

Us: Upslope, Ds: Downslope, TAC: Time-activity curve, CPS: Counts per second. The change in anatomical position of the background had no statistical significant effect on the Us and Ds derived from TAC

Table 3: Baseline profile of the background ROI group

Parameter	Bilateral supraclavicular background	Suprathyroid background	Central brain background	P-value
Us				
Range	0.10–0.29	0.014–0.28	0.10–0.28	0.944
Mean±SD	0.09±0.080	0.10±0.090	0.09±0.080	
Ds				
Range	0.260–0.39	0.150–1.90	0.260–1.90	0.849
Mean±SD	0.75±0.535	0.85±0.548	0.75±0.534	
Min CPS				
Range	2.5–67	3.50–247	2.5–63	0.028
Mean±SD	23.62±19.581	53.87±61.220	18.84±18.66	
Max CPS				
Range	17.85–199	20–317	18–195	0.157
Mean±SD	67.57±53.979	100.4±78.303	60.92±46.099	

SD: Standard deviation, CPS: Counts per second. Minimum CPS of the background pixels in pixel histogram varies with the change in the anatomical position of background region of interest, but this change had not affected the semi-quantitative parameters of time-activity curve

rate, defined by the rate constant k_{21} , and also there is elimination from the peripheral compartment affected by the rate constant k_{10} . Hence, the observed rate of the distribution process is described by the hybrid rate constant α , which is dependent on the three rate constants k_{12} , k_{21} , and k_{10} , as in Equation 17.9. Thus, it is obvious that although k_{12} varies with the tissue composition, the minimum CPS of the background ROI changes with the change in anatomical position. However, it does not affect the overall distribution in the salivary glands as explained by the above-mentioned equation, so the value of U_s is not dependent on k_{12} independently.

$$\alpha = \frac{1}{2} \left[\frac{(k_{12} + k_{21} + k_{10}) + \sqrt{(k_{12} + k_{21} + k_{10})^2 - 4k_{21}k_{10}}}{2} \right] \quad (17.9)$$

Both the minimum counts and the maximum CPS depend on k_{12} , but the maximum count does not depend solely on k_{12} , unlike the minimum CPS because with the progression of time, the central elimination, k_e , becomes the most sensitive determining factor [Figure 10]. Thus, the maximum CPS remains independent of tissue composition only as well as change in anatomical position of background ROI.

This k_e may be written in terms of the micro constants (k_{10} , k_{12} , and k_{21})^[8,9] used in the two-compartment model. This point of equilibrium is unique in that there is one overall elimination rate constant, k_e , just as we saw in the

Table 4: Association between U_s , D_s with minimum CPS and maximum CPS in the whole cohort correlation analysis calculation of Pearson's correlation coefficient

Parameters	Correlation coefficient r	P-value	95% Confidence interval for r
U_s and minimum CPS	0.1325	0.3693	-0.1576–0.4015
D_s and maximum CPS	-0.3972	0.0052	-0.6123–-0.1275
U_s and maximum CPS	0.05237	0.7237	-0.2353–0.3316
D_s and minimum CPS	-0.2471	0.0904	-0.4964–0.3979

D_s : Downslope, U_s : Upslope, CPS: Counts per second. Maximum CPS of background region of interest did not change with the positional change of background, but it affects the D_s inversely in contrast to the U_s that remains largely unaffected by the maximum counts

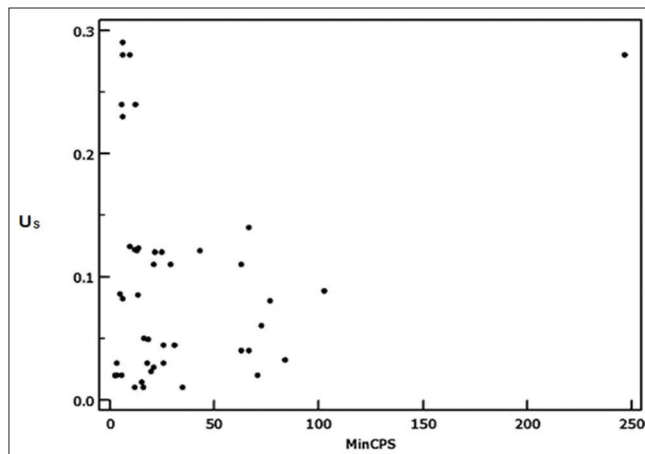


Figure 10: Scatter diagram pattern for upslope data and minimum CPS data

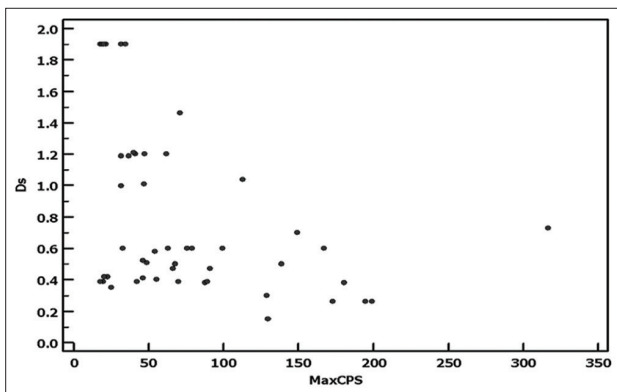


Figure 11: Scatter diagram pattern for Downslope data and maximum CPS data

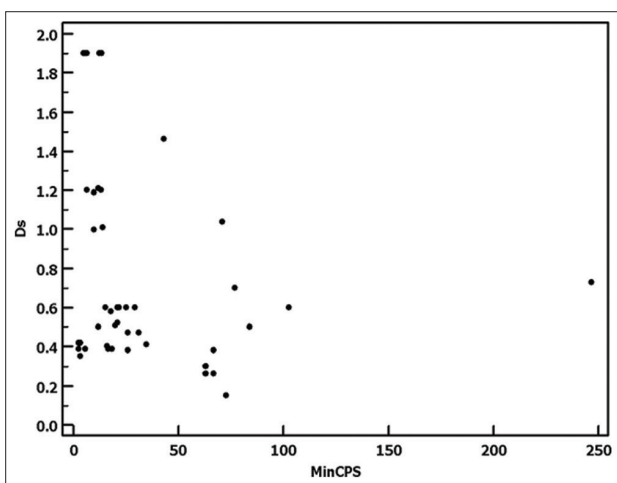


Figure 12: Scatter diagram pattern for Downslope data and minimum CPS data

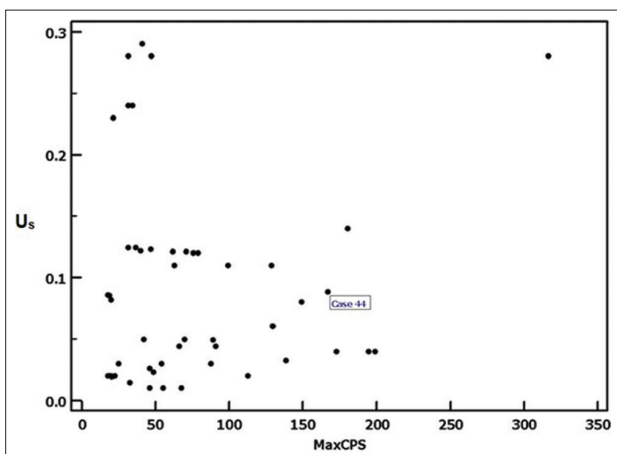


Figure 13: Scatter diagram pattern for upslope data and maximumCPS data

one-compartment model. The smaller hybrid rate constant β is the rate constant for the elimination process as in $\alpha + \beta = \{k_{12} + k_{21} + k_{10}\}$,^[8] hence, it is obvious that tissue-dependent k_{12} , alone is of no importance in calculation of elimination rate constant β .

This might be one of the reasons that maximum CPS of background ROI also does not change with the positional change of background. This is due to developing insignificance of the individual effect of k_{12} with the progression of time. The maximum CPS in background are inversely proportional to maximum CPS of salivary glands, and stimulated secretion index varies with the maximum count in the salivary gland directly. Hence, greater the maximum pixel count of the background less the stimulated D_s index [Figure 11].

CONCLUSION

In salivary scintigraphy, the major important semi-quantitative parameters do not vary with the change in the anatomical position of background but pixel histogram analysis of the background ROI reveals that it is the minimum CPS of the background pixel histogram that varies with the changing position but does not affect the overall quantitative parameters [Figure 10 and 12]. On the contrary, it was observed that the maximum CPS that affects the downslope inversely although U_s remains unaffected [Figure 13 and 11].

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