

# Effects of thyroid hormone replacement on glycated hemoglobin levels in non diabetic subjects with overt hypothyroidism

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## ABSTRACT

**Objective:** Glycated hemoglobin (HbA<sub>1c</sub>) may not accurately reflect the level of glycemia in conditions of altered erythrocyte turnover. Hypothyroidism is one condition associated with sluggish erythropoiesis. To assess changes in HbA<sub>1c</sub> independent of changes in plasma glucose after initiation of thyroxine replacement in patients with overt hypothyroidism. **Materials and methods:** In this prospective longitudinal study carried out in a tertiary care centre, adult non-diabetic patients with overt hypothyroidism recruited between March 2012 to August 2013 were rendered euthyroid on thyroxine. They underwent testing for hemoglobin, HbA<sub>1c</sub>, reticulocyte count, thyroxine, thyrotropin and a standard oral glucose tolerance test, both before and at 3 months after restoration to the euthyroid state. Main outcome assessed was the change in HbA<sub>1c</sub> independent of the change in glucose parameters. **Results:** Thirty eight patients (35 female and 3 male) aged 37.8 ± 10.2 years with overt hypothyroidism (thyroxine 12.6 ± 13.4 ng/mL and thyrotropin -98.1 ± 63.7 µIU/mL respectively) were recruited. While HbA<sub>1c</sub> fell from 5.8 ± 0.7% to 5.6 ± 0.5% (p = 0.009) at 3 months following the correction of hypothyroidism, there were no changes in the fasting and the 2 hr post oral glucose tolerance test glucose (p = 0.67 and 0.56 respectively). The number of patients with dysglycemia diagnosed by HbA<sub>1c</sub> (i.e. HbA<sub>1c</sub> ≥ 5.7%) fell from 25 (65.78%) to 17 (44.7%) after treatment (p = 0.008). There were 7 (18.4%) patients with HbA<sub>1c</sub> ≥ 6.5% at baseline, but this fell to just 4 (10.5%) (p < 0.001) after 3 months of euthyroidism. **Conclusion:** HbA<sub>1c</sub> is not a reliable diagnostic test for diabetes in the presence of hypothyroidism. Arch Endocrinol Metab. 2015;59(6):495-500

## Keywords

Glycated hemoglobin; hypothyroidism; thyroxine

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## INTRODUCTION

The American Diabetic Association has recently approved the use of glyated hemoglobin (HbA<sub>1c</sub>) as a screening as well as diagnostic test for *diabetes mellitus*. A value ≥ 5.7% but < 6.5% was considered to represent pre-diabetes, while a value ≥ 6.5% was considered diagnostic of *diabetes mellitus* (1,2).

The glyated hemoglobin represents the fraction of hemoglobin that undergoes non-enzymatic glycation over the circulatory life span of the erythrocytes (usually 120 days) (3,4). It not only depends on the ambient level of glycemia over the preceding 2-3 months (5), but also on the average period of exposure of the circulatory red blood cells (RBCs) to this glycemia i.e on the erythrocyte turnover in circulation (3,5). Conditions which are associated with a low RBC turnover, with a predomi-

nance of older cells (and a paucity of younger RBCs and reticulocytes) in circulation are associated with a falsely elevated HbA<sub>1c</sub>. Proven examples include iron (6,7) and vitamin B12 deficiency (6), and renal failure (8).

Among the hypoplastic anaemias, an important and common etiological factor is hypothyroidism (9,10). In response to the diminished metabolism and the reduced requirements for oxygen, there is reduced erythropoietin production from the proximal renal tubules (11).

It is conceivable that this reduced erythropoiesis may result in a false elevation of HbA<sub>1c</sub> (12) resulting in some cases in an erroneous diagnosis of pre diabetes or diabetes. We planned to assess changes in patient categorization between euglycemia, prediabetes and *diabetes mellitus* based on HbA<sub>1c</sub> levels, resulting from thyroxine replacement to hypothyroid patients- an aspect not studied before.

## MATERIALS AND METHODS

### Study design

The study was a prospective study conducted in the Department of Endocrinology and Metabolism at a tertiary care centre in India. The study period was from March 2012 to August 2013. Patients aged more than 20 years with a biochemical diagnosis of overt hypothyroidism (i.e. TSH > 15  $\mu$ IU/mL and T<sub>4</sub> < 55 ng/mL) were recruited for the study. Patients satisfying glucose based criteria for *diabetes mellitus* as recommended by the American Diabetes Association (ADA) (13) in 2009, were excluded from the study. Suspected or known case of chronic kidney disease, abnormal haemoglobinopathy, haemolytic disorder, reticulocytosis (reticulocytes > 2.5% of all erythrocytes), bone marrow disorders like aplastic anaemia, myelodysplastic syndrome or recent (< 3 months) blood transfusion were excluded from the study.

Written informed consent was obtained from all patients for participation in the study. The study was cleared by the Institutional Ethics Committee. Weight and height of recruited patients was recorded. Blood samples of the enrolled patients were obtained in the morning between 0800–0900 hr after an overnight fast for fasting plasma glucose (FPG), serum creatinine, haemoglobin (Hb), HbA<sub>1c</sub>, reticulocyte count and serum T<sub>4</sub> and TSH. Patients were categorized on HbA<sub>1c</sub> alone into euglycemia (HbA<sub>1c</sub> < 5.7%), prediabetes (HbA<sub>1c</sub>  $\geq$  5.7 but < 6.5) and *diabetes mellitus* (HbA<sub>1c</sub>  $\geq$  6.5%) based on diagnostic cutoffs of HbA<sub>1c</sub> advocated by the American Diabetes Association (2). Dysglycemia based only on HbA<sub>1c</sub> was defined as HbA<sub>1c</sub>  $\geq$  5.7%, i.e. it included patients with both prediabetes and *diabetes mellitus* diagnosed on HbA<sub>1c</sub>.

Each patient then was given 75 gm anhydrous glucose mixed in 200 mL water and asked to drink slowly over 3 minutes. Repeat samples for plasma glucose were collected after 2 hours. Patients with fasting plasma glucose (FPG)  $\geq$  100 mg/dL were labeled as having impaired fasting glucose (IFG), while those with 2 hrs post OGTT plasma glucose (PGPG)  $\geq$  140 mg/dL were labeled having impaired glucose tolerance (IGT). Prediabetes based on plasma glucose measurements was diagnosed by the presence of either IFG or IGT or both. Patients with FPG  $\geq$  126 mg/dL or PGPG  $\geq$  200 mg/dL were excluded from the study as previously stated, with the intention of excluding patients satisfying glucose based criteria for *diabetes mellitus*.

### Biochemical assays

T<sub>4</sub> was measured by radioimmunoassay (RIA) method by using BRIA MAG 4 kits [Broad of Radiation and Isotope Technology (BRIT), Mumbai, India]. The minimum and maximum detectable limits for serum T<sub>4</sub> was 15–240 ng/mL. TSH was measured by immunoradiometric assay (IRMA) method by using IRMAK-9 kits [Broad of Radiation and Isotope Technology (BRIT), Mumbai, India]. The minimum and maximum detectable limits for serum TSH was 0.15–100 mIU/L. The intra assay and inter assay CV (%) ranges from 2.79–6.92 and 1.80–8.21 respectively. HbA<sub>1c</sub> was analysed using commercially available HPLC kits (D-10 HemoglobinA<sub>1c</sub> Prog, Bio-Rad Laboratories, California, United States). The intra and inter assay CV (%) was 0.78 and 0.52 respectively. Plasma glucose and serum creatinine were measured using autoanalyzer CX5 (Beckman Coulter, California, United States of America). Glomerular filtration rate was calculated using the Cockcroft Gault formula (14) and corrected to 1.73 m<sup>2</sup> BSA. Haemoglobin was estimated on an automated haematology analyzer by colorimetric method (Shenzhen Mindray Biomedical Electronics Co Ltd, Hamburg 20537, Germany). Reticulocytes were counted by a trained observer after staining of peripheral blood smear with a supravital stain (new methylene blue) and expressed as a percentage of total red blood cells.

Patients were then started on thyroxin supplements for 3 months. The dose of the drug was increased periodically (every 4–6 weeks) step wise, based on TSH estimations till the patients were rendered euthyroid, i.e. had TSH between 0.5–5  $\mu$ IU/ml. Once the patient had been restored to the euthyroid state, the patient was allowed to continue the same dose of thyroxin and recalled after three months. After three months of documentation of euthyroidism, the following investigations were repeated as before: serum T<sub>4</sub> and TSH, fasting plasma glucose and post glucose load 2 hr plasma glucose, HbA<sub>1c</sub>, haemoglobin and reticulocyte percentage. Reticulocyte percentage was determined by the same expert, who performed the same determination at baseline.

### Sample size

The study was planned to detect change in HbA<sub>1c</sub> before and after restoration of the euthyroid state with a power of 80% and a significance level at a  $p < 0.05$ . The standard deviation of HbA<sub>1c</sub> ( $\sigma$ ) in the normal population, as represented by the normal healthy control subjects

in the study by Kim and cols. (12) was 0.31%. In that study the correction of hypothyroidism lead to fall of 0.2% in the HbA<sub>1c</sub>. Expecting to demonstrate a similar or greater change in our patients, the minimal sample size required to demonstrate a change ( $\delta$ ) of 0.2% in the HbA<sub>1c</sub> was obtained as given below (15):

$$n = \frac{2 (Z_{\alpha} + Z_{1-\beta})^2 \sigma^2}{\delta^2}$$

where  $Z_{\alpha}$  (coefficient of  $\alpha$  error) assumes a value of 1.96 and  $Z_{1-\beta}$  (coefficient of  $\beta$  error) takes a value of 0.8416 for a statistical power of 80% and at a significance level of  $p < 0.05$  for comparison of means of HbA<sub>1c</sub> before and after the correction of hypothyroidism by a 2 tailed paired t test. Substituting the values of  $\sigma$  and  $\delta$  as mentioned above the required sample size was 37.

### Statistical analysis

Data was recorded on a pre designed proforma and managed using Microsoft Excel 2007 (Microsoft Corp, Redmond, WA). Continuous variables were compared with same parameters measured 3 months after the restoration of euthyroidism using two tailed paired t test with a p value of  $< 0.05$  being considered as significant. Baseline proportions were compared with those measured 3 months after the restoration of euthyroidism by the McNemar  $\chi^2$  test. Statistical software SPSS version 15 (SPSS Inc., Chicago, IL) was used for statistical analysis.

## RESULTS

### Baseline clinical features

A total of 38 consecutive patients with confirmed overt hypothyroidism (i.e TSH  $> 15$   $\mu$ IU/ml and T<sub>4</sub>  $< 55$  ng/mL) were recruited for the study over a period of one and a half years. Baseline patient characteristics are given below in table 1.

### Effect of thyroxine replacement

Patients were re-evaluated 3 months after restoration to the euthyroid state. The recorded changes are given in table 2.

Following correction of T4 and TSH to  $105.7 \pm 36.9$  ng/mL and  $2.5 \pm 3.5$   $\mu$ IU/mL respectively, there was a reduction in the mean body weight by 3.5 kg ( $p = 0.004$ ). Likewise, there was improvement in the hemoglobin from  $11.7 \pm 1.9$  gm/dL to  $12.1 \pm 1.6$  gm/dL ( $p = 0.044$ ). This was accompanied by a rise

in the reticulocyte percentage from  $0.9 \pm 0.6\%$  to  $1.2 \pm 0.7\%$  ( $p = 0.046$ ).

**Table 1.** Baseline characteristics of recruited patients

Patient parameter	Value (n = 38)
Age	37.8 $\pm$ 10.2 years
Female : Male ratio	35: 3
Weight	61.0 $\pm$ 13.2 kg
BMI	25.0 $\pm$ 4.2 kg/m <sup>2</sup>
Number of obese patients (% of total)	20 (46.51%)
Serum creatinine	0.79 $\pm$ 0.20 mg/dL
Estimated GFR	104.93 $\pm$ 29.40 mL/min/1.73 m <sup>2</sup> BSA
Thyroxine	12.6 $\pm$ 13.4 ng/mL
Thyrotropin	98.1 $\pm$ 63.7 $\mu$ IU/mL

BMI: body mass index; GFR: glomerular filtration rate.

Normal values thyroxine: 55-135 ng/mL; thyrotropin 0.5-5.0  $\mu$ IU/mL.

**Table 2.** Comparison of anthropometry, hematological parameters, thyroid profile, glycemic indices and proportion of patients with abnormal glycemic parameters before and 3 months after the correction of hypothyroidism in 38 patients

Parameter	Pre therapy N = 38	Post therapy N = 38	P value
Weight (kg)	61.0 $\pm$ 13.2	57.5 $\pm$ 14.3	0.004
BMI (kg/m <sup>2</sup> )	25.0 $\pm$ 4.2	24.1 $\pm$ 4.3	0.039
T <sub>4</sub> (ng/mL)	12.6 $\pm$ 13.4	105.7 $\pm$ 36.9	$< 0.001$
TSH ( $\mu$ IU/mL)	98.1 $\pm$ 63.7	2.5 $\pm$ 3.5	$< 0.001$
Hb (gm/dL)	11.7 $\pm$ 1.9	12.1 $\pm$ 1.6	0.044
Reticulocyte (as % of total RBCs)	0.9 $\pm$ 0.6	1.2 $\pm$ 0.7	<b>0.046</b>
FPG (mg/dL)	86.8 $\pm$ 11.0	87.5 $\pm$ 9.1	NS
PGPG (mg/dL)	123.4 $\pm$ 35.6	127.0 $\pm$ 28.8	NS
HbA <sub>1c</sub> (%)	5.8 $\pm$ 0.7	5.6 $\pm$ 0.5	<b>0.009</b>
No. (%) of patients with IFG (FPG $\geq$ 100 mg/dL)	4 (10.5%)	5 (13.1%)	NS
No. (%) of patients with IGT (PGPG $\geq$ 140 mg/dL)	11 (29%)	11 (29%)	NS
No. (%) of patients with IFG and /or IGT	12 (31.6%)	15 (39.4%)	NS
No. (%) of patients with HbA <sub>1c</sub> $\geq$ 5.7	25 (65.7%)	17 (44.7%)	<b>0.008</b>
No. (%) of patients with HbA <sub>1c</sub> $\geq$ 5.7 with normal FPG and PGPG	16 (42.1%)	7 (18.4%)	<b>0.035</b>
No. (%) of patients with HbA <sub>1c</sub> $\geq$ 6.5%	7 (18.4%)	4 (10.5%)	$< 0.001$

BMI: body mass index; T<sub>4</sub>: thyroxin; TSH: thyroid stimulating hormone; Hb: hemoglobin; FPG: fasting plasma glucose; PGPG: post glucose tolerance test 2 hours plasma glucose; HbA<sub>1c</sub>: glycated hemoglobin; IFG: impaired fasting glucose; IGT: impaired glucose tolerance; NS: not significant ( $p > 0.05$ ).

Normal values T<sub>4</sub>: 55-135 ng/mL, TSH 0.5-5.0  $\mu$ IU/mL, Hb: 12-14 gm/dL in females and 14-16 gm/dL in males, Reticulocyte (%) 0.5-1.5%. FPG  $< 100$  mg/dL, PGPG  $< 140$  mg/dL, HbA<sub>1c</sub>  $< 5.7\%$ .

## Changes in glycemc indices

Table 2 shows the comparison between the HbA<sub>1c</sub>, FPG and PGPg at baseline and at 3 months after the correction of hypothyroidism. While there was a fall in the HbA<sub>1c</sub> from  $5.8 \pm 0.7\%$  to  $5.6 \pm 0.5\%$  ( $p = 0.009$ ) following the correction of hypothyroidism, there were no corresponding changes in the fasting and the 2 hr post OGTT plasma glucose ( $p > 0.05$ ).

Changes in the proportion of patients with abnormal glycemc indices in OGTT before and after treatment is also shown in table 2. The proportion of patients with IFG and those with IGT did not change ( $p > 0.05$  for both). The proportion of patients with prediabetes based on plasma glucose values (i.e having IFG or IGT or both) also did not change ( $p > 0.05$ ). However the number of patients with dysglycemc diagnosed by HbA<sub>1c</sub> (i.e HbA<sub>1c</sub>  $\geq 5.7\%$ ) fell from 25 (65.78%) to 17 (44.7%) after treatment ( $p = 0.008$ ). Likewise the number of patients with HbA<sub>1c</sub>  $\geq 5.7\%$  but having normal plasma glucose both in the fasting and the 2 hrs post OGTT state (i.e false diagnosis of dysglycemc by HbA<sub>1c</sub>) also fell from 16 (42.1%) to 7 (18.42%) ( $p = 0.035$ ).

No patient fulfilled glucose criteria for diabetes at baseline, due to exclusion of such patients at baseline. Nevertheless there were 7 (18.4%) patients with HbA<sub>1c</sub>  $\geq 6.5\%$  at baseline (representing false diagnosis of DM by HbA<sub>1c</sub>), but this fell to just 4 (10.5%) ( $p < 0.001$ ) after 3 months of euthyroidism.

On multiple linear regression analysis, none of the following parameters: namely the change in the hemoglobin, reticulocyte percentage, body mass index or the fasting or post OGTT plasma glucose were independently predictive ( $p > 0.05$ ) of the change in the HbA<sub>1c</sub> between the baseline and final follow up.

## DISCUSSION

Recently the American Diabetes Association has recommended the use of HbA<sub>1c</sub> for the diagnosis of *diabetes mellitus* (2). However there has always been concern about the use of HbA<sub>1c</sub> in certain conditions, where this does not accurately reflect the level of glycemia. Disorders associated with changes in the RBC turnover can affect the HbA<sub>1c</sub> independently from the glycemc status, by altering the relative proportion of young and old RBCs in circulation i.e the average age of the RBCs (3,4). Thus disorders with reduced RBC turnover and a preponderance of older RBCs in circulation, like iron

deficiency (6,7), vitamin B12 deficiency (6), or renal failure (8), can have a falsely elevated HbA<sub>1c</sub>.

Among the latter group of disorders, one which is widely prevalent is hypothyroidism. Indeed studies in our country show that overt hypothyroidism is found in 3.9% to 10.95% of the general population (16,17). These prevalence rates are much higher than that found in the Western population, where the prevalence of overt hypothyroidism is only 0.3-0.4% of the adult population (18,19). Thus, India seems to be having a much higher burden of hypothyroidism. Thus any impact of hypothyroidism on HbA<sub>1c</sub> is likely to be a bigger issue in India than in the Western countries.

We enrolled newly diagnosed patients with overt hypothyroidism. Those patients with FPG or PGPg in the diabetic range (i.e FPG  $\geq 126$  mg/dL and PGPg  $\geq 200$  mg/dL) were excluded from the study since they would require anti-diabetic medication or therapeutic lifestyle change, in addition to thyroxin treatment – thus introducing a confounding factor of impact of diabetes treatment on HbA<sub>1c</sub>, while studying HbA<sub>1c</sub> alterations with thyroxin replacement. Only patients with normal glucose values or patients with IFG/IGT were thus included. The reason for choosing three months follow up was on account of the approximately 120 days lifespan of the RBCs in circulation (3,4).

Our study showed mild anaemia with a low reticulocyte count at baseline in concordance with other studies (12,20). In our patients, following thyroid hormone replacement, the haemoglobin and the reticulocyte count rose. Likewise Bashir and cols. (20), in a retrospective study showed that as compared to a Hb of  $10.73 \pm 0.86$  gm/dL in untreated primary hypothyroid patients it was  $12.64 \pm 1.33$  in treated primary hypothyroid patients ( $p < 0.001$ ).

Though there was a mean weight loss of approximately 3.5 kg from baseline following the correction of hypothyroidism, the plasma glucose values remained unaltered (Table 2). Further the proportion of patients with dysglycemc states IGT or IFG or prediabetes based on plasma glucose (either IGT and/or IFG) did not change. These findings are similar to what was observed by Kim and cols. (12) who showed that there were no changes observed in FPG or in 1,2 an hydroglycitol (an index of postprandial hyperglycemc) following the correction of hypothyroidism.

Despite the mean FPG and PGPg being normal, the mean HbA<sub>1c</sub> at baseline was already in the prediabetes range. A significant proportion of patients had a false

diagnosis of dysglycemia (42%) or diabetes (18.4%) by HbA<sub>1c</sub>. Thus in hypothyroidism there is a very high false positive rate for the diagnosis of dysglycemia, if HbA<sub>1c</sub> alone is used as the diagnostic test.

This false elevation of HbA<sub>1c</sub> was also demonstrated by Kim and cols. (12) who showed that HbA<sub>1c</sub> in 45 hypothyroid patients was higher than that in control subjects ( $5.54 \pm 0.43\%$  vs.  $5.34 \pm 0.31\%$  in hypothyroid patients and controls respectively;  $p < 0.001$ ), despite the lower level of plasma fasting glucose in the hypothyroid individuals. Christy and cols. (21) selected 30 hypothyroid (TSH > 14 mIU/L) non diabetic (FPG < 100 mg/dL) patients with normocytic normochromic anemia and compared these patients with 30 euthyroid non diabetic patients also with normocytic normochromic anemia. HbA<sub>1c</sub> in the hypothyroid patients was  $6.32 \pm 0.75\%$  vs.  $5.87 \pm 0.46\%$  in the euthyroid group, the difference being statistically significant. Despite all subjects in this study being euglycemic, the odds ratio for HbA<sub>1c</sub> > 6.5% in hypothyroid patients with anemia was 3.16 in comparison to euthyroid persons.

Following correction of hypothyroidism and maintenance of the euthyroid state for up to 3 months, the initially elevated mean HbA<sub>1c</sub> was found to fall to a normal value despite there being no change in the FPG and the PGPG. Similar findings were reported by Kim and cols. (12) in their 30 hypothyroid patients who were resumed on thyroxin replacement. In their study, HbA<sub>1c</sub> fell from  $5.57 \pm 0.26\%$  at baseline to  $5.37 \pm 0.32\%$  1 month after commencing thyroxin replacement; a magnitude of fall similar to that seen in our study.

Change in the HbA<sub>1c</sub> cannot be attributed to the fall in the BMI as there was no significant association between these parameters on multiple linear regression. Moreover there is no mechanism by which the BMI can influence the HbA<sub>1c</sub> other than through the level of glycemia, which has remained unaffected between baseline time point and post 3 months follow up.

Similar reduction of HbA<sub>1c</sub> without attempting to control glucose has also been observed in patients with vitamin B<sub>12</sub> deficiency treated with vitamin B<sub>12</sub> or iron deficiency individuals treated with iron (7) or renal failure patients treated with erythropoietin (8). In all these cases the fall in HbA<sub>1c</sub> was attributed to the appearance of newly formed young RBCs in circulation. In our patients also, this process is reflected in the observed rise in the reticulocyte percentage. However there was no statistical association between the change in the HbA<sub>1c</sub> and the change in the reticulocyte percentage or the

hemoglobin. This may be due to the following reasons. Firstly, though the study is adequately powered to detect changes in HbA<sub>1c</sub> between pre and post treatment time points, it may not be adequately powered to assess causal associations with changes in the reticulocyte percentage. Secondly the reticulocytes only represent the “youngest” red blood cells, but do not fully reflect the entire population of “young” RBCs in circulation. There is currently no satisfactory method to study the average age of the RBCs in circulation at two different points in time, which would have been the ideal tool in this study.

In our study the diagnosis (based on HbA<sub>1c</sub>) of dysglycemia (HbA<sub>1c</sub>  $\geq 5.7\%$ ) made earlier was subsequently changed to euglycemia in nearly one third of the patients initially diagnosed as dysglycemia. Similar reduction was observed in the proportion of patients with the wrong diagnosis (based on HbA<sub>1c</sub>) of dysglycemia (i.e HbA<sub>1c</sub>  $\geq 5.7$  despite normal FPG and PGPG) and *diabetes mellitus*. Thus a very small reduction in HbA<sub>1c</sub> of only 0.2% nevertheless has a significant impact on the HbA<sub>1c</sub> based diagnostic categorization of hypothyroid patients. This aspect has not been studied earlier.

To conclude, levels of HbA<sub>1c</sub> are falsely elevated, out of proportion to the level of glycemia in patients with hypothyroidism. However it is lowered without any change in plasma glucose after correction of hypothyroidism. Consequently false diagnosis of dysglycemia may be made if HbA<sub>1c</sub> alone is used for diagnosis in patients with overt hypothyroidism. Therefore in hypothyroid patients, reliance should be placed only on fasting plasma glucose or the oral glucose tolerance test (OGTT) to diagnose prediabetes or type 2 diabetes.

The study is limited by having only a small number of patients, a relatively short follow up duration of 3 months and by the absence of a control group. A larger study with a longer follow up may be of use to confirm our results.

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