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Evaluation of Vitamin-D Level as an Important Diagnostic Predictor in Progression of Chronic Kidney Disease in Tertiary Care Hospital - A Prevalence Study

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Abstract
Introduction: *This study was destined to find out possible link between progression of Chronic kidney disease (CKD) and vitamin D level which is now very common worldwide specially in third world country like India where scarcity of research made it an emerging public health problem.*
Materials and Methods: *A total of 70 cases of CKD and 30 normal age, sex matched individuals as controls were taken after satisfying inclusion, exclusion criteria. Semi auto-analyzer to estimate serum creatinine and GFR estimation from the serum creatinine value was done by using Modification of diet in renal disease (MDRD) study equation. Specific reagent kit was used to estimate ACR. Estimation of serum Vitamin D (25-OH-D) was done by enzyme linked immunosorbent assay (ELISA). Correlation between serum Vitamin D level with GFR and ACR value were calculated to comment on role of Vitamin D level in progression of CKD. CKD was graded according to Kidney Disease Improving Global Outcomes (KDIGO) 2012 classification by GFR and ACR.*
Results & Conclusion: *71.4% of CKD cases were found to be vitamin D deficient whereas control group showed only 30% prevalence (P<0.001 using Pearson Chi Square test).*
Among the CKD cases there were significant deficiency of Vitamin D level with increasing CKD grade (49.9% in G3a, 53.83% in G3b, 68.42% in G4 and 83.48% in G5) (P<0.05 using Pearson Chi Square test).
Keywords: *Vitamin D, Chronic kidney disease, GFR, ACR, KDOQI.*

Introduction

According to Kidney Disease Outcomes Quality Initiative (K/DOQI) guidelines, chronic kidney disease (CKD) is defined either by reduced kidney function or by the presence or absence of kidney damage - irrespective of etiology.[1] Severity of CKD can be classified in 5 stages – according to the level of glomerular filtration rate and the presence of markers of kidney damage such as (micro-) albuminuria. Progression of CKD may lead to end-stage renal disease (ESRD), requiring renal replacement therapy such as dialysis or transplantation.

According to the latest Endocrine Society guidelines, serum levels of 25(OH)D between 20 and 30 ng/ml indicate vitamin D insufficiency and levels less than 20 ng/ml indicate vitamin D deficiency.[2] Severe deficiency is defined as a 25(OH)D level less than 10 ng/ml. The KDIGO (Kidney disease: improving global outcomes) guidelines recommend that the serum level of 25(OH) D should be maintained over 30 ng/ml in patients of all stages of CKD.[3]

Vitamin D is regarded as prehormone obtained through the diet or via skin synthesis. Then it gone through subsequent activation in a sequential 2-step process, involving first 25-hydroxylation in the liver to produce 25-(OH)vitamin D and then 1-hydroxylation, which recently was thought to occur primarily in the kidney, to produce the active product 1,25 vitamin D or calcitriol.[4,5,6] The 1,25 renal-activated end-product was responsible for all of the effects of the active vitamin D hormone in the body especially to regulate bone and mineral metabolism.[4,7] A more expanded role for 25-(OH) vitamin D was suggested by the ubiquitous existence of the vitamin D receptor in the body,[6,8,9] the presence of at least 800 human genes for which there is a vitamin D response element,[4,6] and the wide distribution of the 1-alpha-hydroxylase in non-renal tissues such as the skin, vascular smooth muscle cells, pancreas, kidney, heart, immune system, intestine and sarcoid tissue.[4,5]

A peripheral autocrine pathway beside classical pathway for activation of 25-(OH) vitamin D to $1,25(OH)_2$ vitamin D exists and results in calcitriol synthesis in a variety of peripheral (non-renal) tissues.[4,7] In fact, it appears that the bulk of daily metabolic utilization of 25-(OH)-vitamin D is via the peripheral autocrine pathway, although its contribution to circulating $1,25\text{-}(OH)_2$ vitamin D is minimal due to immediate local degradation.[4] Calcitriol synthesized in this manner in the cells and tissues that possess these pathways serves as a critical component in the signalling cascades that bridge external stimuli to gene transcription.[4,7] By binding with its intracellular vitamin D receptor (VDR) in these tissues, calcitriol can regulate cellular proliferation and differentiation, inflammation, the immune system and the endocrine system, including renin-angiotensin System (RAS), insulin resistance and lipid metabolism.[7,9]

The discovery of this non-classical pathway (which is also present in renal tissue) has brought new significance to the importance of addressing vitamin D deficiency given the potential role that hypovitaminosis D may play in multiple chronic diseases such as diabetes, chronic infectious processes, hypertension, cardiovascular disease and CKD.[7,8] Vitamin D deficiency is of high prevalence in the general population[4,6,8] and several evidences suggested relation between disproportionately high incidences of hypovitaminosis D in patients with chronic kidney disease (CKD).[4,5,7-11] to an greater extent.[7,8,10]

The non-classical effects of vitamin D may play a relevant role in the mortality and morbidity of patients with CKD, specifically affecting the possible progression of their renal disease[11] and coexisting cardiovascular disease, which is the major cause of death in this population.[8]

In India, there is a paradox that despite adequate sunlight, deficiency of vitamin D is quite prevalent in the general population. This may be explained by clothing habits of Indian population, their pigmented skin, and changing lifestyle with limited outdoor activities.[12] However, limited data are available in patients with CKD stage 5 on dialysis. There has been renewed interest in studying the effects of supplementation with native vitamin D (cholecalciferol) in CKD patients with low 25(OH)D levels.[13,14]

There is still limited data is available about their relationship, and more so from eastern part of India. To fill up this lacuna we have studied prevalence of Vitamin D insufficiency and deficiency in patients of chronic kidney disease attending at outpatient department of our Medical College, which is a prominent Medical College of eastern India and want to predict diagnostic value of Vitamin D3 in respect to progression of CKD.

Materials and Methods

Study Area: Department of Biochemistry, in collaboration with, Department of Medicine, in our Medical College & Hospital, Kolkata.

Study population: Patients admitted at Medicine indoor of our Medical College and Hospital, Kolkata with provisional clinical diagnosis of chronic kidney disease. (GFR<60ml/min/1.73m2).

Sample size: Total eighty (70) samples were taken from review of previous research papers. 30 age and sex frequency matched samples were taken as control.

Sample design

- **Selection of cases –**
- **Inclusion Criteria**
  - Patients admitted at Medicine indoor of our Medical College and Hospital, Kolkata with provisional clinical diagnosis of chronic kidney disease.
  - Age more than or equal to 18 years.
- **Exclusion Criteria**
  - Patients with diagnosed renal malignancy or malignancy in any other parts of body with or without treatment were excluded.
  - Patients with malabsorption syndrome, gastric or small bowel resection were excluded.
  - Patients with severe hepatocellular disease were excluded.
  - Patients on phenytoin, phenobarbital and rifampin were excluded.
  - Non-cooperative patients/ patients not giving consent were excluded.

Selection of control

30 apparently healthy, age and sex matched subjects with no history of chronic kidney disease were recruited from laboratory staffs and their family members constituted the control group in this study.

Each participant was provided with a written informed sheet and blood and urine sample were collected after taking a written informed consent.

Study design

This is a cross sectional non-interventional case control study.

Parameters studied

a) Estimation of serum creatinine in auto analyzer and estimation of GFR from the serum creatinine value by using MDRD study equation.
b) Estimation of urinary albumin and urinary creatinine in auto analyzer and calculating the albumin creatinine ratio.
c) Estimation of serum Vitamin D (25(OH)D) in enzyme linked immunosorbent assay.
d) Correlation between serum Vitamin D level with GFR and ACR value to comment on role of Vitamin D level in progression of CKD.

CKD was graded according to Kidney Disease Improving Global Outcomes 2012 classification by GFR and ACR.[3].

Table 1 CKD grading by GFR and ACR (KDIGO 2012 classification)

Category	GFR (Ml/min/1.73 m2)
G1	>=90
G2	60-89
G3a	45-59
G3b	30-44
G4	15-29
G5	< 15
Category	**ACR (mg/g)**
A1	<30
A2	30-300
A3	>300

Table 2: Classification of 25OH Vitamin D status

Level	ng/ml
Deficient	**<10**
Insufficient	**10-29**
Sufficient	**30-100**
Potential toxicity	**>100**

Correlation of Vitamin D level and above mentioned CKD category was done using specific statistical tools.

Study techniques

The patients were selected first from the Medicine Indoor of our Medical College and Hospital, Kolkata according to the inclusion & exclusion criteria after obtaining consent of the patient in proper consent form. 70 cases were selected randomly from those patients. Detailed history was gathered. After that blood and urine for

biochemical investigations was collected. .The collected data was then analyzed.

Plan for analysis of data

All recorded data was analyzed using standard statistical methods including standard diagrams and graphs. Statistical softwares like IBM SPSS™ and Microsoft excel 2013™ were used for this purpose.

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Conflict of interest: No.

Results

Among the 70 cases mean age was 51.31 + standard deviation of 17.4 with minimum age of 22 year and maximum age of 76 year. Most (38.5%) cases were between 61 to 80 years age group. Among the 30 controls mean age was 46.5 with standard deviation of 16.6. Minimum age was 22 year and maximum age was 67 year. Most (40%) were between age group of 61 to 80 years.

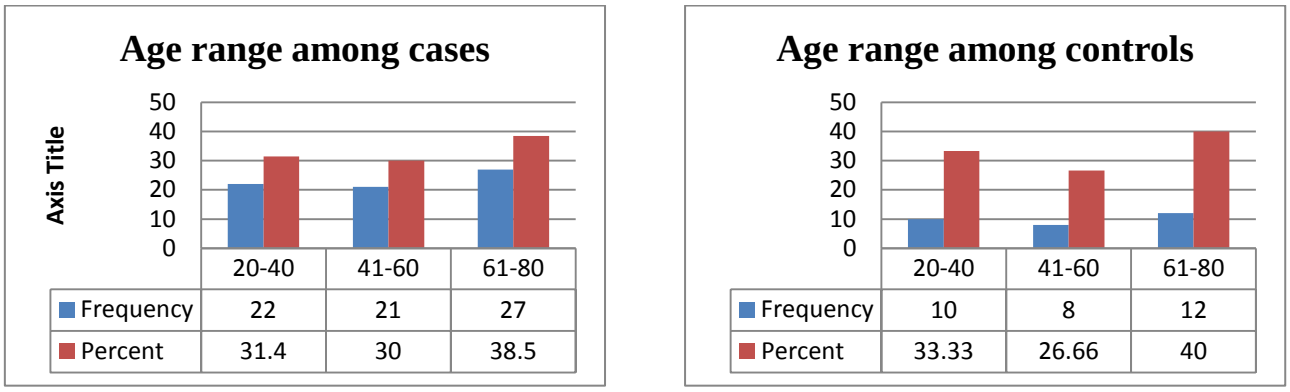


Fig 1: age wise distribution of total 70 cases (left) and 30 controls (right).

Among the 70 cases 63.4% were male and 35.6% were female. Among the 30 controls 65.4% were male and 34.6% were female.

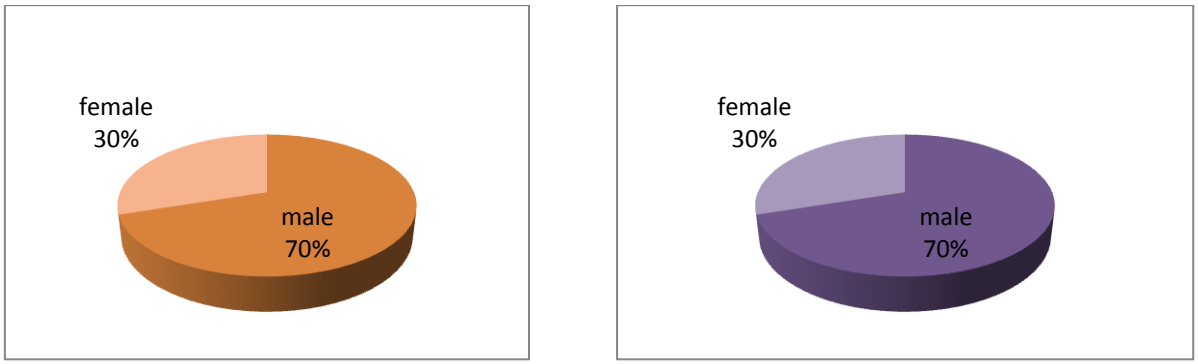


Fig 2: Sex wise distribution among cases (orange pie) and controls (violet pie).

Statistical analysis of Serum creatinine level

Table 2: showing statistical analysis of serum creatinine level in both cases and controls

Serum creatinine level		
	Cases (n=70)	**Control (n=30)**
Mean	4.9	0.84
Median	3.8	0.78
Std. Deviation	2.9	0.23
Minimum	1.49	0.38
Maximum	11.59	1.28

From Table 2, it is observed that serum creatinine level is much higher in CKD patients than control group. Using independent samples t-test for equality of mean we found this difference of creatinine level among cases and controls to be statistically significant ($p<0.001$).

Statistical analysis of Serum Vitamin D level

Table 3: showing statistical analysis of serum Vitamin D level in cases and controls

Serum Vitamin D level						
	N	**Mean**	**Median**	**Std. Deviation**	**Minimum**	**Maximum**
Cases	70	20.48	17.38	12.66	6.63	54.12
Controls	30	50.78	42.86	26.78	19.73	98.14

From Table 3 we can see that serum vitamin D level is much lower in CKD patients than control group. Using independent samples t-test for equality of mean we found this difference of vitamin D level among cases and controls to be statistically significant (p<0.001).

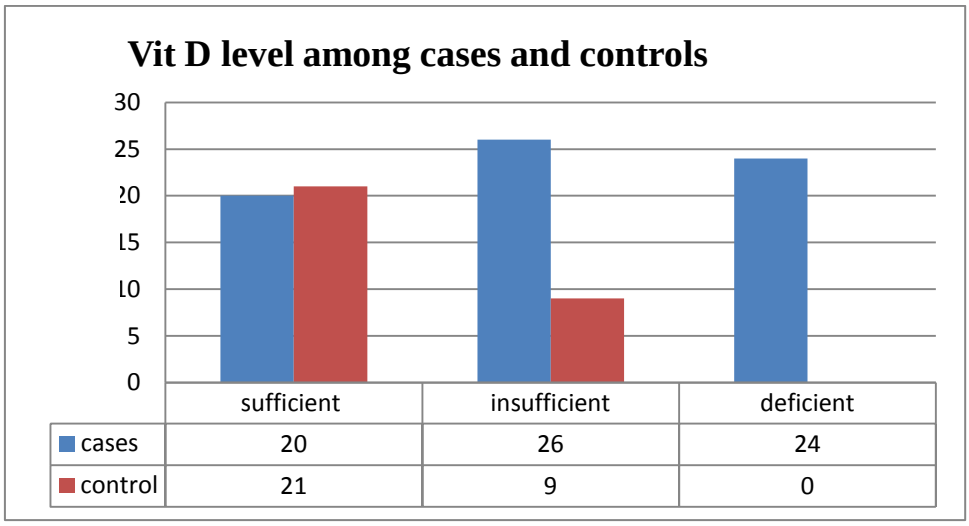


Fig 3: showing distribution of vitamin D level among cases and controls

From the diagram we can see that among the 70 cases 26 (37.14%) had insufficient and 24 (34.28%) had deficient vitamin D level, i.e. among the 70 cases 50 (71.4%) had either insufficient or deficient vitamin D level. Whereas among 30 controls, 21 (70%) had sufficient serum vitamin D level and only 9 (30%) had insufficient vitamin D level. This distribution is found to be statistically significant p < 0.001 using Pierson Chi-square test.

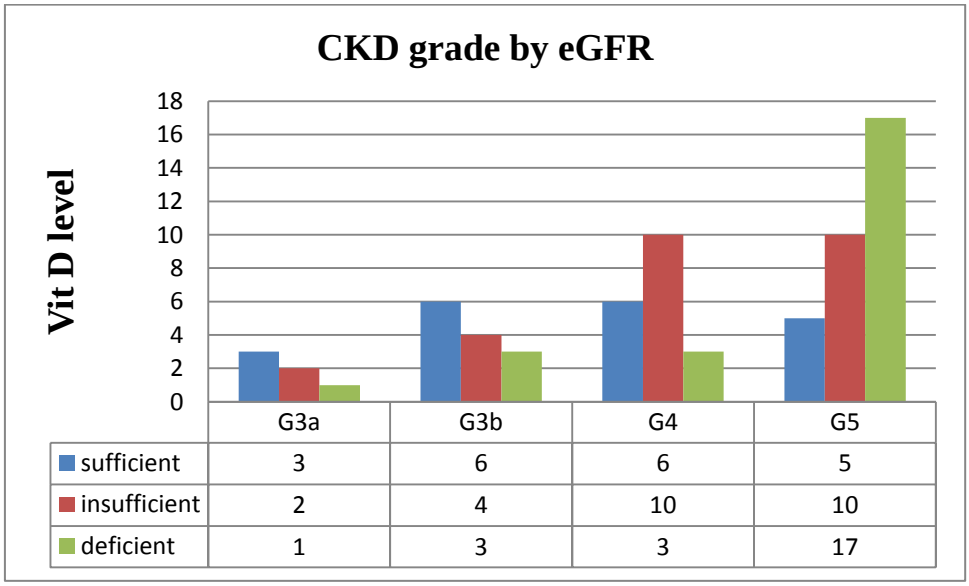
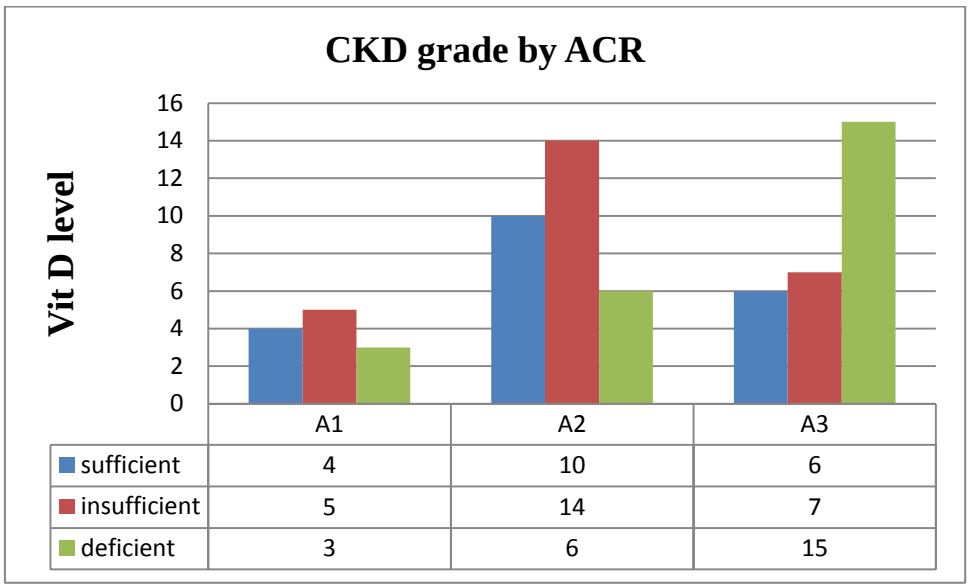


Fig 4: tabulation of CKD grade by eGFR with vitamin D level among cases

Among the 6 Grade 3a CKD patients 2 (33.33%) had insufficient and 1 (16.66%) had deficient vitamin D level, i.e., 49.9% had either vitamin D deficiency or insufficiency. Among the 13 Grade 3b CKD patients 4 (30.76%) had insufficient and 3 (23.07%) had deficient vitamin D level, i.e., 53.83% had either vitamin D deficiency or insufficiency. Among the 19 Grade 4 CKD

patients 10 (52.63%) had insufficient and 3 (15.78%) had deficient vitamin D level, i.e., 68.42% had either vitamin D deficiency or insufficiency. Among the 32 Grade 5 CKD patients 10 (31.25%) had insufficient and 17 (53.13%) had deficient vitamin D level, i.e. 84.38% had either vitamin D deficiency or insufficiency. So we can see that with increasing grade of CKD there is more vitamin D insufficiency or deficiency. When we study correlation and significance of this data using Spearman's rho correlations, the correlation coefficient comes out -0.420, which is statistically significant at the 0.01 level (2-tailed).



	A1	A2	A3
sufficient	4	10	6
insufficient	5	14	7
deficient	3	6	15

Fig 5: Cross tabulation of CKD grade by ACR with vitamin D level among cases

Among the 12 ACR grade 1 patients 5 (41%) had insufficient and 3 (25%) had deficient vitamin D level, i.e. 66.0% had either vitamin D deficiency or insufficiency. Among the 30 ACR grade 2 patients 14 (47%) had insufficient and 6 (20%) had deficient vitamin D level, i.e. 67.0% had either vitamin D deficiency or insufficiency. Among the 28 ACR grade 3 patients 7(25%) had insufficient and 15 (53.57%) had deficient vitamin D level, i.e. 78.57% had either vitamin D deficiency or insufficiency. So, we can say with higher ACR grade there is more vitamin D deficiency or insufficiency. When we study correlation and significance of this data using Spearman's rho correlations, the correlation coefficient comes out -0.289, which is statistically significant at the 0.05 level (2-tailed).

Discussion

Our study observed that vitamin D deficiency (<10ng/ml) or insufficiency (10-29ng/ml) increased with decline in estimated GFR. In recent era, emerging evidence suggest that the non-classical effects of vitamin D may play a relevant role in the mortality and morbidity of patients with CKD, specifically affecting the possible progression of their renal disease and coexisting cardiovascular disease. Our study showed vitamin D deficiency or insufficiency in 71.4% of CKD cases whereas control group showed only 30% prevalence (P<0.001 using Pearson Chi Square test).

Among the CKD cases there were significant deficiency/insufficiency of Vitamin D level with increasing CKD grade (49.9% in G3a, 53.8% in G3b, 68.42% in G4 and 84.38% in G5) (P<0.05 using Pearson Chi Square test).This finding corroborates with Study by Blair D et al[15] described that increases prevalence of 25-vitamin D deficiency with progression of CKD and approaches 80% in stage 5 CKD patients. Study by Mehrotra et al[8] found a significant association

between hypovitaminosis D and mortality in a nationally representative sample across the spectrum of CKD before the need for dialysis. Individuals with serum 25OHD levels < 15 ng/ml had a 1.5-fold higher adjusted risk for all-cause mortality. Our study also infers that albuminuric patients are more likely to have decreased Vitamin D level compared to normo albuminuric patients. Among the ACR grade 1 patients 66.0% had either vitamin D deficiency or insufficiency while among ACR grade 3 patients 78.57% had either vitamin deficiency or insufficiency. When we study correlation and significance of this data using Spearman's rho correlations, the correlation coefficient comes out -0.289, which is statistically significant at the 0.05 level (2-tailed).

One of the hallmarks of nephropathy is albuminuria. Third National Health and Nutrition Examination Survey (NHANES III) [16] demonstrated an inverse relationship between the level of vitamin D and degree of albuminuria. These findings suggest that vitamin D may have anti-proteinuric effects, likely via a RAS angiotensin II-mediated mechanism[11].

The reason for this marked 25-(OH) vitamin D deficiency even in early CKD is likely multifactorial and due in part to nutritional deficiency as well as superimposed increased renal loss of vitamin D binding protein, which occurs as a result of proteinuria, a common occurrence in CKD patients [7,10].

A lot of hypothesis were postulated regarding Vitamin D deficiency in chronic kidney disease in recent years. Since our study shows that vitamin D deficiency is more prevalent in higher grades of chronic kidney disease, we can infer that 1α hydroxylase activity decreases as the CKD grade increases. But a recent study on haemodialysis patients showed that 1, 25 dihydroxy vitamin D level increased after supplementation with nutritional vitamin suggesting that even in end stage renal disease there is enough extra renal 1 α hydroxylase activity to influence serum level [17].As our study results indicated that level of vitamin D decrease as eGFR declines, therefore hypovitaminosis D may be an useful diagnostic tool for assessment of risk of CKD disease progression to dialysis and death.

Conclusion

It is still unknown whether vitamin D deficiency is the cause or only the consequence of various chronic diseases. Since these chronic diseases lack specific treatment or the treatment effects are not curative, strategies for the control of chronic diseases should focus on the prevention. Therefore we need to develop a proper diagnostic tool for timely prediction of CKD disease progression. Because of the high rates of hypovitaminosis D and renal disease progression of CKD to end stage was observed in our study populations, treatment with vitamin D is may halt the progression of the disease. So, we can conclude that vitamin D level can be used as predictor of diagnosing CKD disease process towards progression.

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